EFFECTS OF EXERCISE-INDUCED MUSCLE DAMAGE ON NEUROPLASTICITY AND STIFFNESS

A Thesis
by
JOHN WILLIAM MACKALL

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

AUGUST 2018
Department of Health and Exercise Science

EFFECTS OF EXERCISE-INDUCED MUSCLE DAMAGE ON NEUROPLASTICITY AND STIFFNESS

A Thesis
by
JOHN WILLIAM MACKALL
AUGUST 2018

APPROVED BY:

Alan R. Needle, Ph.D.
Chairperson, Thesis Committee

Herman van Werkhoven, Ph.D.
Member, Thesis Committee

Edward K. Merritt, Ph.D.
Member, Thesis Committee

Kelly J. Cole, Ph.D.
Chairperson, Department of Health and Exercise Science

Michael J. McKenzie, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

EFFECTS OF EXERCISE INDUCED MUSCLE DAMAGE ON NEUROPLASTICITY AND STIFFNESS

John William Mackall
B.S. Salisbury University

Chairperson: Alan R. Needle, Ph.D.

Musculoskeletal injuries are one of the leading causes of disability within the general population. Acutely, injury can cause pain, inflammation, and feelings of stiffness impacting the nervous system as well as the muscle to control the injured segment. However, studying injuries in an acute environment can pose challenges with respect to controls and timing of measurement. Exercise-induced muscle damage (EIMD) is also known to alter muscular stiffness and cause pain acutely; therefore, it was the goal of this thesis to examine if EIMD could describe mechanical and neural changes *in vivo*. Changes in muscle architecture, tendon stiffness, reflexive inhibition, and intracortical inhibition were observed over 72 hours following EIMD. Twelve untrained subjects took part in a muscle damage protocol of eccentric calf contractions (10 sets of 10 repetitions at 75% of one repetition maximum) on an isokinetic dynamometer. They were then tested for pennation angle and fascicle length via ultrasound, tendon stiffness using the tendon displacement method, reflexive inhibition by the Hoffman reflex (H-Reflex), and intracortical inhibition as determined by cortical silent period (CSP).

Testing took place before muscle damage, 10 minutes, 24 hours, and 72 hours after muscle damage. No significance was found in any measure despite a significant increase in pain; however, large effect sizes were observed of decreased CSP at 110% of motor threshold (μ_p^2=0.302) and decreased fascicle length (μ_p^2=0.163). These results though are underpowered for both CSP (p=0.135) and fascicle length (p=0.114). From this, it can be said that resting muscle tone could have been altered. More investigation is needed to confirm these effects. Further, examination across different muscle groups would help to further these conclusions.

Acknowledgments

I wish to thank all individuals who have participated in helping to complete this study. Nothing important gets done alone, and I have had a lot of help along the way. I wish to single out Alan Needle and Herman van Werkhoven who suggested I take on this project and have supported me throughout. I wish to also thank my entire thesis committee who have helped me in so many ways from my writing and being flexible with changes to my methodologies. I wish to also thank lab mates who have helped with the data collection process. Robert Paul and Zachary Ripic, thank you for your help. Thank you as well to the Appalachian State University Office of Student Research for funding in part of this study as well at the Department of Health and Exercise Sciences for the purchase and use of supplies and equipment.

Dedication

This thesis is, first and foremost, dedicated to the Lord and Savior of my life, Jesus Christ. It is in Him; my identity is found. My strength and perseverance throughout this process have come from Him. "For through the law I died to the law so that I might live for God. 20 I have been crucified with Christ and I no longer live, but Christ lives in me. The life I now live in the body, I live by faith in the Son of God, who loved me and gave himself for me." (Galatians 2:19-20 NIV).

Secondly, thank you to my fiancé, Sara Pasma. It has been her constant love and support which has kept me going. From giving up her Sunday afternoons with me so that I could do data collection to cooking meals for us, her love and support has been steadfast.

I also wish to thank my family. My parents were supportive of me choosing to go to Appalachian State University and have stood by me as my career goals and aspirations changed. Their encouragement during my thesis has been instrumental in it being finished. I also wish to single out my Great-Uncle Eugene Ford. It has been his financial generosity that made it possible for me to attend graduate school.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Unintentional musculoskeletal injuries are a leading cause of disability among the general population (Hauret, Jones, Bullock, Canham-Chervak, & Canada, 2010). In addition to the immediate impacts of injury such as pain and limited mobility, long-term injury negatively affects an individual's capacity to perform physical activity and ultimately is a deterrent towards overall health (Anandacoomarasamy & Barnsley, 2005; Maffulli, Longo, Gougoulias, Loppini, & Denaro, 2010). Specifically, joint injury is known to modify neuromechanical function, leading to long-term changes in the morphology and neural control of a segment; however, little is known about the acute effects of joint injury, or how musculotendinous damage affects neural and mechanical properties of the joint. Few studies yet have looked at the acute mechanical or neuromechanical changes as the severity of injury and timing are hard to control. Exercise-induced muscle damage (EIMD), micro-tearing of muscle fibers due to intense exercise, potentially offers a repeatable, controlled model to better understand how pain and stiffness are modified in injury models and explain how the nervous system is affected (Allen, 2001; Baker, 1984).

Musculoskeletal injuries are often associated with decreased joint stiffness, often due to direct damage to stabilizing structures such as ligament (Needle, Kaminski, et al., 2017). Stiffness may be further affected by alterations in sensorimotor control, whereby injury impacts the ability of the dynamic joint stabilizers, such as muscle, to appropriately stress-shield the joint. In fact, this sensory degradation has been proposed to impact central nervous system function by creating a negative feedback loop of altered sensation and inappropriate muscular responses. These changes are theorized to induce

neuroplasticity and subsequently alter sensorimotor function (Needle, Lepley, & Grooms, 2017). What is unknown are the acute effects to neuroplasticity and joint function after injury. Alterations in muscular stiffness is a well-known effect of EIMD and injury, in-turn resulting in decreased range-of-motion and impaired motor control (Clarkson, Nosaka, & Braun, 1992; Hoang, Herbert, & Gandevia, 2007). While it has been well established that EIMD increases muscle stiffness (Hoang et al., 2007; Howell, Chleboun, & Conatser, 1993), whether neural influences have a role in these increases has not yet been investigated.

Tendon stiffness and muscle architecture are known contributors to joint stiffness (Latash & Zatsiorsky, 1993; Singer, 2009). There is reason to believe muscle architecture and tendon stiffness change following EIMD. The micro-tearing which occurs from EIMD damages the z-lines altering parallel elastic component of muscle (Newham, McPhail, Mills, & Edwards, 1983); therefore, it stands to reason that some damage would occur in the series elastic component. Damage to the series elastic component, such as the tendon unit, would alter the stiffness of the joint making gross motor movement difficult as force production of the muscle decreases (Lieber, Roberts, Blemker, Lee, & Herzog, 2017). However, most methods for evaluating tendon stiffness in vivo are indirect (Latash & Zatsiorsky, 1993), thereby limiting the conclusions that can be inferred. Newer methods for determining tendon stiffness such as the tendon-displacement model which directly measures tendon stiffness via ultrasound (Rosager et al., 2002) could provide accurate insight to tendon stiffness alterations. Pennation angle (PA) and fascicle length (FL) are properties of muscle architecture and may influence joint stiffness, and although studies have measured changes in PA and FL after muscle fatiguing (N. M. Thomas,

Dewhurst, & Bampouras, 2015), no study has examined their changes after EIMD. Factors though such as inflammation and potential altered neural output, resulting in muscle tone changes, may acutely alter these measures. Studying these changes following EIMD will give further insight to the mechanical contributions of joint stiffness.

Reflexive inhibition is known to occur following ligamentous injury as damage occurring to the sensory receptors, inflammation, and pain result in arthrogenic muscle inhibition (AMI), a type of reflexive inhibition (McVey, Palmieri, Docherty, Zinder, & Ingersoll, 2005). AMI describes the altered communication between the joint and nervous system and is associated with degraded spinal reflexes of the affected joint (McVey et al., 2005). Type III and Type IV afferent sensory fiber activation decreases the excitability of the alpha motor neuron pool causing AMI. The greater the inhibition the less excitable the muscle is to reflexive stimulus, thereby decreasing the ability to activate the muscle (Palmieri-Smith, Villwock, Downie, Hecht, & Zernicke, 2013). What is not yet clear is if this same response can occur due to damage to Type I and Type II afferent fibers. EIMD would be more likely to cause damage to Type I and Type II afferents with minimal to no damage to Type III and Type IV afferents with similar increases in nociception. The degree of reflexive inhibition can be determined by using the Hoffmann reflex (H-reflex) in which electrical stimuli are used to evoke direct responses (M-waves) and reflexive responses (H-waves) from the muscle. The ratio of the maximal H-wave to the maximal M-wave allows the investigator to observe the reflexive abilities of the muscle.

Intracortical inhibition resulting from altered outputs of the brain may occur due to pain following injury and subsequently contributes to muscle stiffness and resting muscle tone (Needle, Charles, et al., 2013). The afferent influx secondary to pain may

result in a decrease in Gamma aminobutyric acid (GABA)-related activity in the cortex would increase descending drive to the muscle, resulting in greater muscle tone and stiffness. This GABA-response to pain has only been observed in ligamentous injury and CNS dysfunctions such as stroke or dystonia (Hallett, 2011). EIMD would then be a novel approach in which to observe this response. The cortical silent period (CSP) represents a period of relative muscular silence following a motor evoked potential induced from transcranial magnetic stimulation. The length of this period is proportional to the amount of GABA activity in the brain and has been associated with the amount of joint stiffness. (Catano, Houa, & Noel, 1997; Kimberley et al., 2009; Trompetto et al., 2012; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999).

By understanding the neural and mechanical contributions behind EIMD, researchers and practitioners will be able to develop better methods to minimize and treat injury. Previous research has established injury-induced neuroplasticity in chronic injury models, as well as EIMD-induced muscle stiffness changes, but no study has before sought to link these properties in a single cohort in vivo. These results will help to illustrate the mechanical and neural contributions of EIMD which may be relayed back to injury. Furthermore, by examining these properties concurrently over time from the onset of damage, the understanding of musculotendinous pathology may be enhanced to improve treatments and minimize performance deficits. We therefore aim to achieve these goals by pursuing the following specific aims:

Specific Aim 1: To concurrently examine changes in tendon stiffness and muscle architecture over 72 hours following EIMD.

Hypothesis 1.1: Tendon stiffness will increase at all time points following EIMD.

Hypothesis 1.2: Pennation angle will increase and fascicle length will decrease at all time points following EIMD.

Specific Aim 2: To examine changes to reflexive and intracortical inhibition over 72 hours following EIMD.

Hypothesis 2: Reflexive inhibition will increase and cortical disinhibition will occur following EIMD.

Chapter 2: Review of Literature

2.1. Introduction

Exercise induced muscle damage (EIMD) is a widely studied topic. From its initial descriptions in 1900 by Hough, there have been numerous studies conducted as to its nature and cause. The purpose of this review is to describe what EIMD is, the competing mechanistic theories as to its occurrence, and its effect on tendon stiffness, PA and FL, reflexive and intracortical inhibition. This review will also draw connections between the etiologies of EIMD and musculotendinous injury and how EIMD can be viewed as a model of musculotendinous injury.

2.2. Exercise Induced Muscle Damage

2.2.1. Definitions

EIMD is the phenomena resulting from eccentric muscle exercise or active lengthening of muscle. It occurs primarily in unaccustomed people and includes a myriad of symptoms. These include delayed onset muscle soreness (DOMS) (Armstrong, 1984), damage to the sarcomeres (Morgan & Allen, 1999) and extra-cellular matrix (ECM), decreases in muscle force production, increases in joint passive stiffness, and onset of muscle inflammation (Clarkson et al., 1992; Hough, 1900). Hough, in 1900, described his unaccustomed subjects as becoming sore days after the activity along with a marked decrease in the force producing capability of the muscle in this same range of time. He hypothesized that the muscles in these subjects became damaged by rupture of the muscles themselves causing these symptoms (Hough, 1900). Since then, many studies have been conducted resulting in competing mechanistic theories as to what causes these wide arrays of effects.

2.2.2. Differences between Muscle Fatigue and Damage

EIMD is distinct and different from muscular fatigue. Hough first described fatigue by showing that tetanic contractions caused soreness during and immediately following exercise. However, soreness did not occur days later in tetanic contractions as was observed with concentric and eccentric contractions (Hough, 1900). Newham et al established that the eccentric portion of muscle activity that caused soreness and damage (Newham et al., 1983). Subjects performed eccentric contractions on one leg and concentric contractions on the contralateral leg. The biopsies taken showed damage to the muscle fibers on the leg that performed eccentric activity while subjects also reported DOMS on that same leg as well. The researchers were able to conclude that active muscle lengthening caused EIMD.

Fatigue differs in that it is largely neuromuscular and is caused by isometric/tetanic activity and concentric exercise (Hough, 1900; Newham et al., 1983). Further, fatigue is not associated with damage to the muscle.

2.3. Mechanisms

2.3.1. Popped Sarcomere Theory

The most prevalent theory in the literature to describe EIMD is termed the "popped sarcomere" theory. When active muscle is stretched beyond a yielding point, the force-lengthening relationship of the sarcomere is shifted to longer lengths (Katz, 1939). This would indicate that the sarcomeres were stretched to a yielding point which in turn increased its resting length. The occurrence of this resting length shift indicated damage to the structure. In essence, the sarcomere "pops." Friden et al. took muscle biopsies of human subjects who had performed eccentric exercise and showed evidence of damage to

individual sarcomeres (Friden, Sjostrom, & Ekblom, 1981). The term "popped sarcomere" was coined by D.L. Morgan (Morgan, 1990). Morgan assumes a non-uniform sarcomere model (Morgan, Mochon, & Julian, 1982), where when activated and stretched, the sarcomeres with the least amount of overlap of thick and thin filaments will "pop" or rather shear first (Morgan & Proske, 2004). The process of sarcomere "popping" continues as the muscle lengthens until the rising passive tension accounts for the falling active tension (Morgan & Proske, 2004). This leads to the damage of ECM (Lieber, Loren, & Friden, 1994; Stauber, Clarkson, Fritz, & Evans, 1990). There is evidence for this occurring supported by many studies. Animal and human models have shown that sarcomeres, as well as the ECM, become damaged following eccentric activity (Crameri et al., 2007; Friden et al., 1981; Lieber et al., 1994; Newham, Jones, Ghosh, & Aurora, 1988; Gordon L Warren, Ingalls, Lowe, & Armstrong, 2002; Whitehead, Weerakkody, Gregory, Morgan, & Proske, 2001; Yu, Carlsson, & Thornell, 2004).

2.3.2. Excitation-Contraction Coupling Failure

The other prevalent mechanism of force reduction is excitation-contraction (EC) coupling failure. Experiments conducted using caffeine supplementation showed there was no difference between force production of damaged and control muscle. Caffeine induces release of calcium from the sarcoplasmic reticulum (SR) allowing for an abundance of calcium within the sarcomere. This leads to the conclusion that there is a reduction of stimulus to release calcium from the SR due to EIMD; thereby, decreasing muscle force generation (G. L. Warren et al., 1993).

A marked reduction in the amount of calcium in the sarcomere has been observed in eccentrically damaged mouse muscle (G. L. Warren et al., 1993). Further, a paper by Warren et al published in 2002 stated that EC coupling failure could account for 75% of reduction in force producing capabilities in the first three days following EIMD with the remaining 25% being due to structural damage. After this time, structural damage is the primary cause of force reduction (Gordon L Warren et al., 2002).

2.3.3. Overlap of Popped Sarcomere and EC Coupling Mechanisms

It is important to realize that these mechanisms are not mutually exclusive of each other but rather inter-related. Morgan has since been able to show that the popped sarcomere theory can help explain why EC coupling failure occurs. Damage to the t-tubules has been shown to account, at least in portion, for the reduction in calcium release from the SR (Morgan & Proske, 2004). It is likely that these competing theories harmoniously describe the occurrence of force production loss.

2.4. Musculotendinous Stiffness

2.4.1. Measures of Stiffness

Stiffness in classical mechanics describes the deformation of a spring in relation to the force applied. Hooke's Law describes this relationship: $F = -kx$. F is force applied, x is deformation, and k is the spring constant. The spring constant is an inherent property of the spring. The negative term indicates that it is a restoring force with an equilibrium point. Muscles and tendons are often described by their spring-like characteristics. Terms such as muscle stiffness, tendon stiffness, and joint stiffness are often seen in the scientific literature to describe the spring-like characteristics. It should be noted that there are different measures of stiffness such as quasi-stiffness which is

independent of time and apparent stiffness in which measurement is taken at equilibrium while ignoring some of the physical nature of the spring (Latash & Zatsiorsky, 1993).

Tendons have a unique stiffness constant due to uniform nature of the tissue. Tendon is composed of collagenous tissue unlike muscle which is made up of various proteins and involve these proteins actively contracting. This allows tendon to be modeled as a spring.

Muscle too is often modeled as spring despite the properties described above. The Hill muscle model demonstrates that muscle has elastic components, a series elastic component (SEC) and parallel elastic component (PEC), which accompany the contractile component (CC) (Close, 1972; Hill, 1950). Methods to evaluate the elastic components, SEC and PEC, of muscle in vivo involve the subjects relaxing the tested muscle to remove the CC and is often called passive stiffness. When evaluating stiffness of active muscle, in methods such as free-oscillation (Fukashiro, Noda, & Shibayama, 2001), the PEC is often ignored as it is far more compliant than the SEC. It is also assumed that sarcomere length remains uniform (Latash & Zatsiorsky, 1993). These factors make it difficult to attribute a stiffness constant to muscle.

Joint stiffness describes the combined actions of muscle and tendon to control movement around a joint. This is typically done in a passive manner. The joint is manipulated throughout a set range of motion by a dynamometer while the muscles relax. This reduces the effects of the CC allowing for the elastic components to be measured (Latash 1993). Further, it can be used to evaluate the contribution of reflex loops to stiffness regulation.

2.4.2. EIMD and Stiffness

A noted characteristic of EIMD is the increase in stiffness of the joint (Armstrong, 1984; Hoang et al., 2007; Howell et al., 1993; McHugh et al., 1999; Morgan, 1994). Studies have shown that passive stiffness measures rise after EIMD for at least 48 hours post damage (Hoang et al., 2007; Howell et al., 1993; Whitehead et al., 2001). The length of time to return to baseline levels differs among the literature, but there seems to be a consensus that normal passive stiffness is restored within about a week post EIMD. The rise in passive stiffness has been attributed to EC coupling mechanism (Morgan et al., 1982; Gordon L Warren et al., 2002). When damage occurs the sarcomeres are unable to regulate the flow of calcium from the SR. Calcium then stays in the sarcomere causing increased sarcomere cross-bridging at rest. This increases tension of the CC which further puts stress on the SEC. Overall, this causes a rise in joint stiffness.

2.4.3. Measuring Tendon Displacement to determine Tendon Stiffness

The literature is lacking is in its ability to distinguish tendon stiffness from muscle stiffness. Previous models used involve many assumptions and does not directly use Hooke's Law to determine a spring constant. With the use of an isokinetic dynamometer and ultrasound, force and tendon displacement can be directly measured. According to the methods of Rosager et al. the ultrasound probe is fixed to the muscle-tendon junction and an electrical goniometer is fixed to the ankle. A passive range of motion is performed and a displacement versus angle graph obtained. This is to account for passive tendon displacement and allows for adjustment to the contraction displacement curve (Rosager et al., 2002). Moment arm of the ankle must be estimated in order to obtain the actual force production of the muscle. This model allows then for the spring constant to be obtain

using $k = \frac{Force}{displacement}$. This method has been used in other studies (Arampatzis, Karamanidis, Morey-Klapsing, De Monte, & Stafilidis, 2007; Kubo et al., 2007) as well but never in an EIMD model.

2.5. Effects on Muscle Architecture

Few studies have been performed examining EIMD and changes in muscle architecture measurements of fascicle length and pennation angle. Fascicle length is the total length of the fascicle which can be measure via ultrasound, and pennation angle is the angle formed between a fascicle and the aponeurosis of the muscle. The relationship of fascicle length and pennation angle is indicative of the function of pennate muscle. Increases in fascicle length at rest can mean that there is a lack of filament overlap; thereby, decreasing force production, assuming no sarcomeres have been added in series (Fukunaga, Kawakami, Kuno, Funato, & Fukashiro, 1997; Herzog, Read, & Ter Keurs, 1991; Maganaris, 2003). Likewise, if fascicle length decreases, there is more filament overlap meaning force production has increased; however, the force production will decrease when the fibers become too shortened (Fukunaga et al., 1997; Rassier, MacIntosh, & Herzog, 1999; Woittiez, Huijing, & Rozendal, 1983).

At rest, the higher the pennation angle the larger the physiological cross-sectional area (PCSA) of the muscle. A larger PCSA indicates that there are more sarcomeres in parallel with each other; thus, increasing the active force production capability of a muscle. Pennation angle is also affected by the pull of sarcomeres. This indicates that as the fascicle, and thereby sarcomeres, shorten, the pennation angle increases. Therefore, pennation angle is interdependent on fascicle length (Azizi & Roberts, 2014).

No studies have looked at the effects of EIMD on pennation angle and fascicle length. However, a recent study looked at pennation angle and fascicle length after isometric fatigue in the gastrocnemius medialis of humans. They found that both fascicle length increased and pennation angle decreased following isometric fatiguing (N. M. Thomas et al., 2015). This would indicate that the force production capability of the muscle had decreased immediately following fatigue. Another recent study proved that during eccentric activity, fascicles lengthen in order for there to be a greater contribution of the tendon to the stretch of the muscle thereby decreasing the possibility of damage to contractile tissue (Hoffman, Cresswell, Carroll, & Lichtwark, 2014). Neither of these studies have shown the changes in fascicle length and pennation angle following damage.

2.6. Neurological Influences of Injury

2.6.1. Arthrogenic Muscle Inhibition

Feedback mechanisms from the muscle to the nervous system provide reflex arcs. These reflexes are responsible for resting muscle tone and motor control. Following damage to any of the structures involved results in an arthrogenic muscle response. Arthrogenic muscle response results in either inhibition, which is a decrease in joint control, or facilitation, which is an increase in joint control. In order to quantify the activity of these reflexes, the H-Reflex method is used. H-Reflex tests the excitability of the alpha-motor neuron. Two distinct responses, H-Wave and M-Wave are seen from the test. The H-Wave represents the signal sent from the spinal cord to the corresponding muscle. The M-Wave is the initial twitch of the muscle due to direct stimulation from the testing apparatus. A greater H-Wave indicates facilitation; whereas, a lesser H-Wave indicates inhibition. The maximum of both waves is also compared in the H:M ratio. A

smaller ratio suggests that there are motor-neurons available for recruitment which are not being used. So, the greater the ratio the more inhibition indicated.

It has been shown in ligamentous injury that there is reflexive or AMI (McVey et al., 2005; Needle et al., 2014). Mechanoreceptor damage, pain, and inflammation shut down the reflex arc decreasing the number of motor units recruited. There is no evidence yet as to whether this occurs in musculotendinous injury. The etiologies are similar evidenced by inflammation, pain, and increased joint stiffness but has yet to be tested.

2.6.2. Intracortical Inhibition

Intracortical inhibition is known to play a key role in the maintenance of muscle tone and stability. Intracortical inhibition describes the inhibitory pathways in the brain which suppress unwanted movement. Therefore, inhibitory pathways have an important role in maintaining proper neuromuscular function (Heroux & Tremblay, 2006; Needle, Palmer, Kesar, Binder-Macleod, & Swanik, 2013; Trompetto et al., 2012).

Intracortical inhibition can be quantified by measuring CSP using TMS and EMG. CSP is the time between a motor-evoked potential (MEP) and the return to normal motor function. The MEP is facilitated by using TMS, and the resulting motor function measured by EMG. TMS generates focused B-fields which pass through the skull, exciting the motor neurons in the brain. This causes the neurons to fire creating movement in the corresponding muscles. EMG electrodes placed on these muscles measure the resulting electrical activity. After a TMS stimulus, a spike in muscle electrical activity can be seen called a MEP. Following the MEP normal electrical activity resumes. The time period between the MEP and normal electrical activity is the CSP.

CSP is used primarily to assess people with neurological diseases such as cerebral palsy. From these studies it has been shown that a longer CSP indicates more inhibition and less joint stiffness, and shorter CSP indicates less inhibition and greater joint stiffness(Needle, Palmer, et al., 2013). Ligamentous injury has also been shown to alter CSP. EIMD is known to cause inflammation and increase joint stiffness like ligamentous injury. The onset of inflammation and increase in stiffness has been attributed to both damage of the SR function and sarcomeres. It has not been determined what changes if any occur in muscle tone regulation via the cortex.

2.7. Conclusions

EIMD is a commonly occurring phenomena that effects almost anyone who exercises. The active lengthening of a muscle causes damage to the sarcomeres as well the ECM of the muscle resulting in pain and inflammation. These are shared characteristics with ligamentous injury; however, it is unknown how musculotendinous injury alters reflexive and intracortical inhibition and how that correlates to the changes in stiffness. Using EIMD as a model of musculotendinous injury should allow for insights to be made on this front.

Chapter 3: Methods

3.1. Experimental Design

This study utilized a repeated measures design. The independent variables were the timing in relation to muscle damage protocol (Pre, Post. 24 Hours, and 72 Hours). The dependent variables were tendon stiffness, FL, PA, H_{max}:M_{max}, and CSP. See Figure 1 for a flowchart of study procedures.

3.2. Subjects

Twelve untrained individuals, ages 18-35, were recruited for this study. Subjects were untrained meaning they had not been engaged in a training regimen for at least the prior three months. Subjects were healthy enough to engage in weight lifting activities which was determined by the completion of a Physical Activity Readiness-Questionnaire (S. Thomas, Reading, & Shephard, 1992) form prior to beginning the study. Exclusion criteria included pregnant individuals due to potential health risks, those with current ankle injuries or a history of leg fractures and surgeries, or who regularly took anti-inflammatory medications (i.e. ibuprofen, acetaminophen, or naproxen) as these drugs alter the muscle damage response. Individuals had to also complete the TMS screening questionnaire (Rossi et al., 2009) to ensure the safety of the subject. The TMS exclusion criteria included metal or electronic implant, history of seizure, concussion within the past 6 months, currently pregnancy or being treated for a psychiatric or neurological disorder. All subjects provided Appalachian State University-approved informed consent (16-0256).

3.3. Pain Scale

Subjects were asked to complete a pain assessment form. The form asked the subjects to put a vertical mark along a 10-cm horizontal line indicating the amount of pain in the lower leg while walking, while standing, and while sitting. Below the line was a visual analog scale of facial expressions to help the subject know where to draw the line (See Appendix). The form was completed prior to the start of all visits as well as the end of visit one.

3.4. Determining Muscle Architecture

Subjects were prone on an isokinetic dynamometer (Computer Sports Medicine Inc., Stoughton, MA) with a 10 MHz B-mode ultrasound probe (Telemed Echo Blaster 128, Lithuania) placed to the medial gastrocnemius. The probe was placed at 30% the difference between the popliteal fold and the lateral malleolus as shown in Figure 2 (Kawakami, Ichinose, & Fukunaga, 1998). The subject's foot was placed with the bottom flat on the dynamometer footplate with the foot making a 90-degree angle with the lower leg. This was confirmed by using a goniometer. A high-resolution image was taken with the ultrasound (Figure 3). Analysis of this image was done using Kinovea software (Kinovea for Windows, Version 0.8.15, Kinovea.org). Pennation angle was measured at the smallest angle made between a fascicle and the deep aponeurosis. An average of three angles was taken from a single image. Fascicle length was estimated by measuring the thickness of the muscle. Two measurements from a single image were taken and averaged. Then, along with average pennation angle, fascicle length was calculated by the equation:

$$\frac{Muscle\ Thickness}{\sin(pennation\ angle)} = Fascicle\ Length$$

3.5. Tendon Stiffness

Three methods were performed to determine the moment arm. Anthropometric measurements of the tested leg were taken to determine moment arm of the ankle. Distance from the lateral malleolus to the Achilles' tendon midline was measured as well as from the medial malleolus to the Achilles' tendon midline using calipers. This was done to correct for perspective error of the pictures. Pictures of medial and lateral view of the bare foot were taken. The foot was placed on a box with a ruler on the side to give scale (Figure 4). Marks were made on the lateral and medial malleoli. The lower leg was at a 90-degree angle with the foot, and the lateral and medial edges of the foot were aligned with the reference block. Two pictures were taken, lateral and medial, and the moment arm was said to be the mean of these two calculations. This measurement was done once at the beginning of testing and only at the baseline measures session. These measurements were used in a custom MATLAB (MathWorks, Natick. MA) software to obtain moment arm.

The moment arm was also measured using a tendon excursion method. Two different techniques (MA1 and MA2) of this method were done to check for differences. For muscle architecture, Ultrasound probe was fixed to the muscle tendon junction of the medial gastrocnemius using a custom foam holder and zinc oxide tape to ensure the probe stayed immobile. The subject was prone on an isokinetic dynamometer with the foot flat against the footplate. Straps were used to cover the top of the foot to ensure the foot stayed flat (Figure 5). An electro-goniometer (Biometrics, Newport, UK) was attached at the ankle in the sagittal plane as a check to see if the ankle angle was the same as the angle given by the dynamometer (Figure 6). The dynamometer then moved the ankle

through a 20-degree range of motion at five degrees per second. The foot started at 10 degrees of dorsiflexion, moved to 10 degrees of plantarflexion, and then returned to 10 degrees of dorsiflexion. For MA2, A second trial was performed, this time the ankle was moved through the same range of motion five times. On the fifth pass, data was collected on all instruments. Tracking of the muscle tendon junction was performed using Kinovea (Kinovea for Windows, Version 0.8.15, Kinovea.org) software. The moment arm was determined by using circular dynamics. The tendon excursion method models the ankle as a circle. To determine the radius of a circle, the change in angle and displacement must be known. Tendon excursion is the displacement term, and the change in angle is determined by the goniometer and dynamometer over five degrees of plantarflexion to five degrees of dorsiflexion. The moment arm is the resulting radius (Fath, Blazevich, Waugh, Miller, & Korff, 2010) (see below equation).

$$moment\ arm = \frac{displacement\ of\ tendon}{angular\ ankle\ excurion}.$$

Once the moment arm calculations were performed, subjects then began performing maximal volitional isometric contractions (MVIC) with three minutes in between each trial to measure tendon stiffness. Ultrasound was kept in the same location from moment arm testing. The probe was in video mode recording at 30 frames per second. Data from the dynamometer, goniometer, and ultrasound were all time synched offline for analyses.

Tendon displacement was determined by tracking the muscle-tendon junction using Kinovea analysis. Force was obtained by dividing the torque output of the dynamometer by the moment arm of the ankle and then plotted against displacement. Stiffness was determined at 50% - 100% of total force output and over the whole force output. The resultant slope is the stiffness value. Values were reported for all five

techniques of moment determination (MA1, MA1 with electro-goniometer correction, MA2, MA2 with electro-goniometer correction, and manual calculation).

3.6. Hoffman Reflex

Participants were instrumented with electromyography (EMG) sensors on the tibialis anterior (TA), soleus (SOL), and medial gastrocnemius (MG). Each muscle was palpated, shaved, cleaned with an alcohol swab, and abraded to ensure a quality signal (Basmajian, 1967). H-reflex was acquired using a Digitimer DS7AH stimulator (Digitimer LLC, Hertfordshire, UK) with a bar electrode applied behind the knee. Low intensity pulses were used to find the correct spot behind the knee just before the sciatic nerve bifurcates in the popliteal fossa. Brief electrical pulses of one millisecond were applied beginning at a low intensity and gradually increased by two mA until a maximal response was observed from the muscles. The direct muscle activation (M-wave, 10-40ms) and the reflexive response (H-wave, 50- 14 100ms) was identified and peak-to-peak values were extracted. Electromyography data was collected at 2000 Hz. The ratio of maximal H-wave to maximal M-wave served as a measure of reflexive excitability (Needle et al., 2014).

3.7. Cortical Silent Period

For measurement of CSP, participants kept the EMG sensors on from H-Reflex testing. Magnetic stimuli were delivered using a Magstim 200-2 Magnetic Stimulator with a double-conical coil (MagStim LTD, Wales, UK). Participants were seated in a chair with a tight-fitting cap on the head and provided earplugs to wear throughout testing (Figure 7). After familiarization with the procedures of TMS, magnetic stimuli were gradually applied 1cm anterior and lateral to the vertex of the skull until observable

motor responses were seen in the legs. This intensity was used to find the "hotspot". Stimuli were applied every 5 seconds as the coil was moved within a 5cm radius to pinpoint the location that a pulse generated the largest motor response in the leg muscles. This location was designated the hotspot. Next, the resting motor threshold (the exact magnetic intensity enough to induce a muscular contraction) was pinpointed by applying 50-60 stimuli (with 5 seconds between each) at varying randomized intensities ranging from below the motor threshold to above a maximal response to generate a stimulus-response curve. Resting motor threshold was then determined by finding were the response increased by 10% of maximum (Needle, Palmer, et al., 2013).

TMS was delivered with the subject voluntarily contracting their muscles at 10 percent of maximal effort with augmented feedback provided by the investigators. This is a light level of contraction designed to allow for calculation of the CSP. Ten pulses of 90%, 110% and 130% (30 pulses total) of the resting motor threshold were applied at the hotspot as continuous muscle activity was recorded.

CSP was calculated using custom LabVIEW software (National Instruments, Austin, TX). A series of student's *t*-tests were used to compare EMG activity which had been logarithmically-transformed and normalized to a 4-ms window of pre-stimulus activity. Cursors were placed on a plot and the investigator confirmed the locations were accurate. The researcher then took the difference of the time from the return of voluntary muscle contraction and subtract from it the time at the end of the MEP (Nilsson, Panizza, & Arieti, 1997).

3.8. Muscle Damage Protocol

To begin the muscle damage protocol, a working one repetition maximum had to be performed to determine the level of intensity of the protocol. All instrumentation on the subject was removed, and they then laid prone on the dynamometer with their foot fixed on the footplate with straps. Subjects then performed one MVIC at 20 degrees of plantarflexion to determine maximal force at the end range of motion as this is the weakest position for the exercise. Using 75 percent of the MVIC, subjects performed ten sets of ten repetitions of single leg eccentric calf raises on the dynamometer while pushing maximally against the footplate. Repetitions only utilized the plantar flexor muscles with the concentric portion being one second and the eccentric portion being three seconds. Torque threshold for the concentric portion was set at 10 foot-pounds. Each repetition consisted of the subject moving from 10 degrees of dorsiflexion to 20 degrees of plantarflexion and back to dorsiflexion (Figure 8). One minute of rest was be given between sets. The dynamometer ensured a full range of motion was done for each repetition. The eccentric torque limits of the dynamometer were lessened by 5 foot-pounds a set as fatigue was observed by practitioner so all completed 100 repetitions.

3.9. Statistical Analyses

Statistical analyses were performed using IBM SPSS software. PA, FL, tendon stiffness, and CSP were assessed with a one-way analysis of variance (ANOVA) with time as the within-subjects factor. Time had four levels: pre, post, 24 hours post, and 72 hours post. H-Reflex was assessed using a factorial ANOVA because there are two independent variables, time (4 levels) and muscle (3 levels). The a priori significance level was set at 0.05. Partial eta-squared was used to assess the effect size (0.01 ~ small,

0.06 ~ medium, 0.14 ~ large) (Cohen & Cohen, 1983). Further, Fisher's least significance difference comparisons was used to find post hoc differences between levels of the independent variables in the case of significant main or interaction effects.

Chapter 4: Results

4.1. Subjects

Untrained males (n = 9) and females (n = 3) participated in the current study (age: 22.4 ± 2.7 years; body mass: 82.6 ± 19.8 kg; height: 176.3 ± 6.9 cm). Six of these subjects performed the TMS measures due to an adverse event, which occurred during testing. The testing leg was determined by a coin flip in which eight subjects performed the protocol on the right leg and four subjects on the left leg.

4.2. Pain Scale

Pain values are presented in Table 1. For pain while walking there was a significant main effect of time ($F_{[3,33]} = 4.280$, $p = 0.012$, $\mu_p^2 = 0.280$). From post-hoc comparisons, pain while walking was greater at 24-hours than at baseline ($p = 0.007$). Pain while walking at 24-hours was also significantly greater than at 72-hours ($p = 0.041$). All other times were non-significant from each other. For pain while standing there was not a significant effect of time ($F_{[3,33]} = 1.218$, $p = 0.319$, $\mu_p^2 = 0.100$). For pain while sitting data violated Mauchly's test of sphericity ($W = 0.231$, $p = 0.011$); and therefore, a Greenhouse-Geisser adjustment to the degrees of freedom was used. There was not a significant effect of time ($F_{[1.938,21.270]} = 1.330$, $p = 0.281$, $\mu_p^2 = 0.180$).

4.3. Muscle Architecture

Values of PA and FL are presented in Table 2. For FL there was no significant effect of time ($F_{[3,33]} = 2.139$, $p = 0.114$, $\mu_p^2 = 0.163$). For PA there was no significant effect of time ($F_{[3,33]} = 0.734$, $p = 0.5374$, $\mu_p^2 = 0.048$).

4.4. Moment Arm

Moment arm values are presented in Table 3. For moment arm, there was no significant effect of time ($F_{[3,33]} = 0.346$, $p = 0.793$, $\mu_p^2 = 0.030$). The effect of technique violated sphericity ($W = 0.272$, $p = 0.028$). Using the Greenhouse-Geisser correction there was a significant difference between techniques ($F_{[2.679,29.474]} = 76.368$, $p < 0.001$, $\mu_p^2 = 0.874$). The interaction of technique and time also violated sphericity ($W = 0.000$, $p = 0.002$). The Greenhouse-Geisser Correction was used and no significance was found ($F_{[1.662,44.958]} = 0.407$, $p = 0.775$, $\mu_p^2 = 0.036$).

Comparisons showed that electro-goniometer correction for MA1 and MA2 were significantly greater than MA1 without electro-goniometer correction with $p = 0.002$ and $p = 0.007$ respectively. Further, they were also significantly greater than MA2 without electro goniometer correction with $p = 0.009$ and $p = 0.002$ respectively. The anthropometric determination of moment arm was done only once at the onset of testing and does not have the level of time. Using pairwise comparisons this determination of moment was significantly greater than all the four other techniques all with $p < 0.001$.

4.5. Tendon Stiffness

Tendon Stiffness was determined using the force production range and over 50% - 100% of maximum force production with all 5 techniques of moment arm being used. There was no main effect of time for any determination of tendon stiffness. All means, standard deviations, F-values and P-values are reported in Table 4. Average maximum force from MVIC trails were also computed. All means, standard deviations, F-values, and P-values are reported in Table 5.

4.6. Reflexive Inhibition

Descriptive statistics for reflexive inhibition can be seen in Figure 9. For H-Reflex, the interaction between muscle and time violated sphericity ($W = 0.001$, $p < 0.001$). Greenhouse-Geisser correction was used with the interaction between muscle and time did not yield significant ($F_{[1.717,17.170]} = 0.758$, $p = 0.605$, $\mu_p^2 = 0.070$). Within muscle, SOL was greater than both TA ($p = 0.032$) and MG ($p < 0.001$). The effect of time was not significant ($F_{[3,30]} = 0.231$, $p = 0.874$, $\mu_p^2 = 0.023$). The effect of muscle was significant ($F_{[2,20]} = 9.268$, $p = 0.001$, $\mu_p^2 = 0.481$).

4.7. Intracortical Inhibition

CSP was only performed on six subjects as there was an adverse event from TMS which occurred during testing. Descriptive statistics of CSP can be seen in Figure 10. For CSP at the 130% level, the effect of time was not significant ($F_{[3,12]} = 0.624$, $p = 0.613$, $\mu_p^2 = 0.135$). For CSP at the 110% level, the effect of time was also not significant ($F_{[3,15]} = 2.165$, $p = 0.135$, $\mu_p^2 = 0.302$).

Chapter 5: Discussion

5.1. Introduction

The purpose of this study was to examine the changes of muscle architecture, tendon stiffness, reflexive inhibition, and cortical inhibition over 72 hours following a single bout of EIMD in the triceps surae. It was hypothesized that FL and PA would increase, tendon stiffness would increase, reflexive inhibition would increase, and intracortical inhibition would decrease after EIMD. Despite the increases in pain, there were no significant changes to muscle architecture, tendon stiffness, reflexive inhibition, or intracortical inhibition. FL and CSP though were bordering on significance with large effect sizes suggesting that resting motor tone may have increased, although a larger sample would be required to draw this conclusion. These results indicate that acute changes in stiffness due to EIMD may be a result of the neurological changes as opposed to mechanical, but further investigation is needed before any consensus is reached.

5.2. Muscle Architecture

PA and FL were assessed to quantify muscle architecture following EIMD as they can serve as markers for muscle tone and stiffness. The triceps surae are 3 pennate muscles, with the degree of pennation proportionate to force output and muscle tone (Maganaris, 2003; Singer, 2009). FL is associated with several factors, such as the number of sarcomeres in series, the filament overlap (Hodges, Pengel, Herbert, & Gandevia, 2003), or the amount of fluid levels (Bakke et al., 1996). Knowing that muscle stiffness increases following EIMD (Clarkson et al., 1992), it was predicted that PA would increase and FL would decrease following EIMD. To our knowledge, PA and FL have not been assessed previously in EIMD models. The results did not show statistically

significant changes in either measure. FL though is nearing significance ($p = 0.114$) with a large effect size ($\eta^2 = 0.163$) indicating FL may have decreased and resting muscle tone and muscle stiffness increased. A study done in 2015 looked at muscle architecture in fatigued muscles and showed that FL increased and PA decreased (N. M. Thomas et al., 2015) indicating that acute exercise can alter muscle architecture. The difference here though is that this is a fatiguing model whereas the present study is a damage model. Another study showed that FL increased acutely two hours following eccentric muscle contractions (Hoffman et al., 2014). This study may have looked at damage but the times of assessment were different from the present study which could explain the difference in results. The fact that PA did not change suggests that EIMD is possibly independent from what happens to FL. The possible decrease in FL may mean the MG is more contracted following EIMD indicating increased resting muscle tone and stiffness. These are novel findings and deserve further investigation.

5.3. Tendon Stiffness

Tendon stiffness is also a novel measure with EIMD. Muscle stiffness is known to increase following EIMD based upon findings of previous studies (Clarkson & Sayers, 1999; Hoang et al., 2007; Whitehead et al., 2001; Yanagisawa, Sakuma, Kawakami, Suzuki, & Fukubayashi, 2015). Using the muscle-tendon junction displacement method of tendon stiffness has not been done with EIMD prior to this study limiting our ability to understand the relative components of the CC, PEC, and SEC components to stiffness changes. The micro-tearing that is known to occur in muscle due to EIMD was thought to affect tendon as well; therefore, it was predicted that tendon stiffness would increase

following EIMD due to more pull from the increased muscular stiffness and resting muscle contraction.

To use the muscle-tendon junction displacement method, it was important to first determine the moment arm of the muscle. Moment arm was determined three different ways: two utilizing tendon-excursion and one determined from anthropometric measurements. The first technique of these muscle-tendon junction methods was a single rotation method where only one range of motion of the ankle was performed, and the ultrasound recording the entire duration. The second technique was five rotations of the ankle with ultrasound data being recorded on the fifth full range of motion. No differences were found between MA1 and MA2 lending to the concurrent validity of these techniques and shows that repeated passive motion does not affect the outcome of the muscle-tendon junction method. These methods were also performed at each timepoint for all subjects. No change in the moment arm was found due to EIMD indicating that the methods have some reliability and are unaffected by EIMD. The third method involved anthropometric measurements and picture analysis and was only performed at the start of day one of testing. The photo method was significantly different from the muscle-tendon junction displacement methods though the results did yield values similar to other studies (Scholz, Bobbert, van Soest, Clark, & van Heerden, 2008) indicating that the obtained values were concurrently valid. Overall, no one method is better than the other in the context of this study as the moment arm is simply a scalar of the overall force value, meaning if the moment arm value is consistent and reliable it will not drastically alter the force calculation.

Despite the increase found in pain, there was no change in any calculation of tendon stiffness. As stated previously other studies showed increases in muscle stiffness further supporting the original hypothesis that tendon stiffness would too increase (Howell et al., 1993; McHugh et al., 1999). These results indicate previous findings that have shown stiffness increases were likely mediated by muscle and not tendon.

One of the most interesting findings was the lack of force deficit, which is of the hallmarks of EIMD (Allen, 2001; Clarkson et al., 1992), despite changes in pain. The selected muscle damage protocol could be the reason for the unobserved force decrement but that would imply that EIMD did not occur. Our muscle damage protocol was based on other isokinetic study designs which yielded both pain and damage (Brown, Day, & Donnelly, 1999; Deschenes et al., 2000; Eston, Finney, Baker, & Baltzopoulos, 1996) indicating EIMD should have occurred. It should be noted thought, that these models utilized the quadriceps muscle group instead of the triceps surae group; however, both muscle groups are pennate and motion was limited to one plane ensuring reliability of damage. Therefore, lack of the force deficit could be due to the untrained nature of the subjects. Many studies utilize untrained subjects for EIMD without familiarization (Brown et al., 1999; Eston et al., 1996; Hoffman et al., 2014; Nosaka & Clarkson, 1995); however, a lack of experience with exercise and with the isokinetic dynamometer might have contributed small amount to the lack of the force deficit. Despite the movement being simple and familiar to subjects, perhaps familiarizing them to the feel of the isokinetic dynamometer is warranted in future studies. One other note should be made regarding pennate muscle damage. A recent study has shown that the gearing nature of pennate muscles may protect them from damage (Azizi & Roberts, 2014). A fusiform

muscle model such as the biceps brachii may be more appropriate and increase the amount of damage.

5.4. Reflexive Inhibition

Reflexive inhibition describes the segmental ability to excite the motor neuron pool, with greater H:M ratios indicating a more excitable alpha motor neuron pool. H-Reflex allows for measurement of the excitability of the alpha motor neurons as well as Type I and Type II afferent fibers. Ligamentous injury alters AMI, with increased type III and IV afferent activity secondary to capsular damage and swelling consistently observed to increase reflexive inhibition (Palmieri-Smith et al., 2013). This study tested to see if altered activity of Type I and Type II afferent fibers can elicit a similar increase in reflexive inhibition. EIMD should cause alter activity to the Type I and Type II pathways; therefore, it was predicted that reflexive inhibition would increase due to muscle damage and altered sensory feedback from Type I and Type II afferents (McVey et al., 2005; Palmieri-Smith et al., 2013) as well as AMI response from pain (Palmieri-Smith, Hopkins, & Brown, 2009). The results showed no change in reflexive inhibition following EIMD, and even though pain was present it was not enough to disrupt the motor neuron pool. The damage which should occur as a result of EIMD then is not enough to cause AMI and possible alterations to Type I and Type II afferents are not enough to cause AMI either. It may be concluded then that muscle damage and pain may not acutely change the inhibition of the spinal reflexes or cause AMI.

5.5. Intracortical Inhibition

CSP is a measure of the GABAergic inhibition inside of the brain and is thought to alter resting motor tone of the muscle as well as muscle stiffness, the shorter the silent

period, the less inhibition is present resulting in increases to muscle tone as well as muscle stiffness. Long term injury is known to increase inhibition in the long term leading to disuse and decreased ability to activate the muscle. The effects of acute injury though are less known as it is harder to study. Acute injury is thought to decrease the level of GABAergic activity due to increased sensory input from the periphery, including pain (Needle et al., 2014; Needle, Palmer, et al., 2013). Further, long term muscle tone dysfunctions such as dystonia, which reflect increased resting muscle tone, have shown decreased inhibition (Hallett, 2011). So, based upon injury findings and the known muscle stiffness changes of EIMD (Hoang et al., 2007; McHugh et al., 1999), it was predicted that intracortical inhibition would decrease following EIMD.

CSP did not change at the 130% stimulation levels following EIMD in the present study. There is though a large effect size at the 110% stimulation level ($\eta^2 = 0.302$), but it is underpowered to find ($p = 0.135$). It would then be improper to conclude there is no CSP change. Especially since due to an adverse event, only six subjects of these data were obtained; therefore, a larger sample size then may yield significance. It is important to note as well that the trend at the 110% stimulation level is a decrease from baseline potentially supporting the hypothesis that pain and acute injury lends itself to disinhibition. The results seen in FL also may support the change in CSP. A shorter FL would indicate that the muscle is more contracted due to more overlap within the sarcomeres. All this would indicate a potential change in resting motor tone. Therefore, muscle damage may trigger a change in resting muscle tone and intracortical inhibition. More data is needed to determine if there is a real effect.

5.6. Conclusions

The data here suggests that EIMD may be responsible for changes in intracortical inhibition and resting muscle tone although more data is needed to support this assertion. It also suggests that the effects of EIMD such as feelings of stiffness and force changes is likely mediated in part by neurological changes. The lack of mechanical changes seen in this study seem to support this conclusion. More research is needed to confirm this link. A lack of AMI as well suggests that EIMD models offer differences from ligamentous injury. Type III and Type IV afferent fibers likely do not cause the same response as damage to Type I and Type II afferents.

The pain that was found in the present study does indicate that some form of damage did take place in the subjects, although the degree to damage may be questionable. Future models may be encouraged to track markers of inflammation and muscle damage to observe a potential covariate to these neuromechanical changes. There are improvements that could be made to this model such as using fusiform muscles and trained subjects which may yield better results. Further, it would be prudent to measure joint stiffness or muscle stiffness to observe the relative contributions of muscle, tendon, and capsule towards perceived increases in stiffness.

References

Allen, D. G. (2001). Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiologica Scandinavica, 171*(3), 311-319. doi:10.1046/j.1365-201x.2001.00833.x

Anandacoomarasamy, A., & Barnsley, L. (2005). Long term outcomes of inversion ankle injuries. *British Journal of Sports Medicine, 39*(3), e14; discussion e14. doi:10.1136/bjsm.2004.011676

Arampatzis, A., Karamanidis, K., Morey-Klapsing, G., De Monte, G., & Stafilidis, S. (2007). Mechanical properties of the triceps surae tendon and aponeurosis in relation to intensity of sport activity. *Journal of Biomechanics, 40*(9), 1946-1952. doi:10.1016/j.jbiomech.2006.09.005

Armstrong, R. B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and Science in Sports and Exercise, 16*(6), 529-538.

Azizi, E., & Roberts, T. J. (2014). Geared up to stretch: pennate muscle behavior during active lengthening. *Journal of Experimental Biology, 217*(Pt 3), 376-381. doi:10.1242/jeb.094383

Baker, B. E. (1984). Current concepts in the diagnosis and treatment of musculotendinous injuries. *Medicine and Science in Sports and Exercise, 16*(4), 323-327.

Bakke, M., Thomsen, C. E., Vilmann, A., Soneda, K., Farella, M., & Møller, E. (1996). Ultra sonographic assessment of the swelling of the human masseter muscle after static and dynamic activity. *Archives of Oral Biology, 41*(2), 133-140. doi:10.1016/0003-9969(95)00135-2

Basmajian, J. V. (1967). Electromyography: Its Structural and Neural Basis. In G. H. Bourne & J. F. Danielli (Eds.), *International Review of Cytology* (Vol. 21, pp. 129-140): Academic Press.

Brown, S., Day, S., & Donnelly, A. (1999). Indirect evidence of human skeletal muscle damage and collagen breakdown after eccentric muscle actions. *Journal of Sports Sciences, 17*(5), 397-402. doi:10.1080/026404199365911

Catano, A., Houa, M., & Noel, P. (1997). Magnetic transcranial stimulation: clinical interest of the silent period in acute and chronic stages of stroke. *Electroencephalography and Clinical Neurophysiology, 105*(4), 290-296.

Clarkson, P. M., Nosaka, K., & Braun, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and Science in Sports and Exercise, 24*(5), 512-520.

Clarkson, P. M., & Sayers, S. P. (1999). Etiology of exercise-induced muscle damage. *Canadian Journal of Applied Physiology, 24*(3), 234-248.

Close, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews, 52*(1), 129-197. doi:10.1152/physrev.1972.52.1.129

Cohen, J., & Cohen, P. (1983). *Applied Multiple Regression/correlation Analysis for the Behavioral Sciences*: L. Erlbaum Associates.

Crameri, R. M., Aagaard, P., Qvortrup, K., Langberg, H., Olesen, J., & Kjaer, M. (2007). Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *Journal of Physiology, 583*(Pt 1), 365-380. doi:10.1113/jphysiol.2007.128827

Deschenes, M. R., Brewer, R. E., Bush, J. A., McCoy, R. W., Volek, J. S., & Kraemer, W. J. (2000). Neuromuscular disturbance outlasts other symptoms of exercise-induced muscle damage. *Journal of the Neurological Sciences, 174*(2), 92-99. doi:10.1016/S0022-510X(00)00258-6

Eston, R. G., Finney, S., Baker, S., & Baltzopoulos, V. (1996). Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. *Journal of Sports Sciences, 14*(4), 291-299. doi:10.1080/02640419608727714

Fath, F., Blazevich, A. J., Waugh, C. M., Miller, S. C., & Korff, T. (2010). Direct comparison of in vivo Achilles tendon moment arms obtained from ultrasound and MR scans. *J Appl Physiol (1985), 109*(6), 1644-1652. doi:10.1152/japplphysiol.00656.2010

Friden, J., Sjostrom, M., & Ekblom, B. (1981). A morphological study of delayed muscle soreness. *Experientia, 37*(5), 506-507.

Fukashiro, S., Noda, M., & Shibayama, A. (2001). In vivo determination of muscle viscoelasticity in the human leg. *Acta Physiologica Scandinavica, 172*(4), 241-248. doi:10.1046/j.1365-201x.2001.00866.x

Fukunaga, T., Kawakami, Y., Kuno, S., Funato, K., & Fukashiro, S. (1997). Muscle architecture and function in humans. *Journal of Biomechanics, 30*(5), 457-463.

Hallett, M. (2011). Neurophysiology of dystonia: The role of inhibition. *Neurobiology of Disease, 42*(2), 177-184. doi:10.1016/j.nbd.2010.08.025

Hauret, K. G., Jones, B. H., Bullock, S. H., Canham-Chervak, M., & Canada, S. (2010). Musculoskeletal injuries description of an under-recognized injury problem

among military personnel. *American Journal of Preventive Medicine, 38*(1 Suppl), S61-70. doi:10.1016/j.amepre.2009.10.021

Heroux, M. E., & Tremblay, F. (2006). Corticomotor excitability associated with unilateral knee dysfunction secondary to anterior cruciate ligament injury. *Knee Surgery, Sports Traumatology, Arthroscopy, 14*(9), 823-833. doi:10.1007/s00167-006-0063-4

Herzog, W., Read, L., & Ter Keurs, H. (1991). Experimental determination of force—length relations of intact human gastrocnemius muscles. *Clinical Biomechanics, 6*(4), 230-238.

Hill, A. V. (1950). The series elastic component of muscle. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 137*(887), 273-280.

Hoang, P. D., Herbert, R. D., & Gandevia, S. C. (2007). Effects of eccentric exercise on passive mechanical properties of human gastrocnemius in vivo. *Medicine and Science in Sports and Exercise, 39*(5), 849-857. doi:10.1249/MSS.0b013e318033499b

Hodges, P. W., Pengel, L. H., Herbert, R. D., & Gandevia, S. C. (2003). Measurement of muscle contraction with ultrasound imaging. *Muscle and Nerve, 27*(6), 682-692. doi:10.1002/mus.10375

Hoffman, B. W., Cresswell, A. G., Carroll, T. J., & Lichtwark, G. A. (2014). Muscle fascicle strains in human gastrocnemius during backward downhill walking. *J Appl Physiol (1985), 116*(11), 1455-1462. doi:10.1152/japplphysiol.01431.2012

Hough, T. (1900). Ergographic Studies in Muscular Fatigue and Soreness. *Journal of the Boston Society of Medical Sciences, 5*(3), 81-92.

Howell, J. N., Chleboun, G., & Conatser, R. (1993). Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *Journal of Physiology, 464*, 183-196.

Katz, B. (1939). The relation between force and speed in muscular contraction. *Journal of Physiology-London, 96*(1), 45-64. doi:DOI 10.1113/jphysiol.1939.sp003756

Kawakami, Y., Ichinose, Y., & Fukunaga, T. (1998). Architectural and functional features of human triceps surae muscles during contraction. *J Appl Physiol (1985), 85*(2), 398-404. doi:10.1152/jappl.1998.85.2.398

Kimberley, T. J., Borich, M. R., Prochaska, K. D., Mundfrom, S. L., Perkins, A. E., & Poepping, J. M. (2009). Establishing the definition and inter-rater reliability of cortical silent period calculation in subjects with focal hand dystonia and healthy controls. *Neuroscience Letters, 464*(2), 84-87. doi:10.1016/j.neulet.2009.08.029

Kubo, K., Morimoto, M., Komuro, T., Tsunoda, N., Kanehisa, H., & Fukunaga, T. (2007). Influences of tendon stiffness, joint stiffness, and electromyographic activity on jump performances using single joint. *European Journal of Applied Physiology, 99*(3), 235-243. doi:10.1007/s00421-006-0338-y

Latash, M. L., & Zatsiorsky, V. M. (1993). Joint Stiffness - Myth or Reality. *Human Movement Science, 12*(6), 653-692. doi:Doi 10.1016/0167-9457(93)90010-M

Lieber, R. L., Loren, G. J., & Friden, J. (1994). In vivo measurement of human wrist extensor muscle sarcomere length changes. *Journal of Neurophysiology, 71*(3), 874-881. doi:10.1152/jn.1994.71.3.874

Lieber, R. L., Roberts, T. J., Blemker, S. S., Lee, S. S. M., & Herzog, W. (2017). Skeletal muscle mechanics, energetics and plasticity. *Journal of Neuroengineering and Rehabilitation, 14*(1), 108. doi:10.1186/s12984-017-0318-y

Maffulli, N., Longo, U. G., Gougoulias, N., Loppini, M., & Denaro, V. (2010). Long-term health outcomes of youth sports injuries. *British Journal of Sports Medicine, 44*(1), 21-25. doi:10.1136/bjsm.2009.069526

Maganaris, C. N. (2003). Force-length characteristics of the in vivo human gastrocnemius muscle. *Clinical Anatomy, 16*(3), 215-223. doi:10.1002/ca.10064

McHugh, M. P., Connolly, D. A., Eston, R. G., Kremenic, I. J., Nicholas, S. J., & Gleim, G. W. (1999). The role of passive muscle stiffness in symptoms of exercise-induced muscle damage. *American Journal of Sports Medicine, 27*(5), 594-599. doi:10.1177/03635465990270050801

McVey, E. D., Palmieri, R. M., Docherty, C. L., Zinder, S. M., & Ingersoll, C. D. (2005). Arthrogenic muscle inhibition in the leg muscles of subjects exhibiting functional ankle instability. *Foot and Ankle International, 26*(12), 1055-1061. doi:10.1177/107110070502601210

Morgan, D. L. (1990). New Insights into the Behavior of Muscle during Active Lengthening. *Biophysical Journal, 57*(2), 209-221. doi:Doi 10.1016/S0006-3495(90)82524-8

Morgan, D. L. (1994). An explanation for residual increased tension in striated muscle after stretch during contraction. *Experimental Physiology, 79*(5), 831-838.

Morgan, D. L., & Allen, D. G. (1999). Early events in stretch-induced muscle damage. *J Appl Physiol (1985), 87*(6), 2007-2015. doi:10.1152/jappl.1999.87.6.2007

Morgan, D. L., Mochon, S., & Julian, F. J. (1982). A quantitative model of intersarcomere dynamics during fixed-end contractions of single frog muscle fibers. *Biophysical Journal, 39*(2), 189-196. doi:10.1016/S0006-3495(82)84507-4

Morgan, D. L., & Proske, U. (2004). Popping sarcomere hypothesis explains stretch-induced muscle damage. *Clinical and Experimental Pharmacology and Physiology, 31*(8), 541-545. doi:10.1111/j.1440-1681.2004.04029.x

Needle, A. R., Baumeister, J., Kaminski, T. W., Higginson, J. S., Farquhar, W. B., & Swanik, C. B. (2014). Neuromechanical coupling in the regulation of muscle tone and joint stiffness. *Scandinavian Journal of Medicine and Science in Sports, 24*(5), 737-748. doi:10.1111/sms.12181

Needle, A. R., Charles, B. B. S., Farquhar, W. B., Thomas, S. J., Rose, W. C., & Kaminski, T. W. (2013). Muscle spindle traffic in functionally unstable ankles during ligamentous stress. *J Athl Train, 48*(2), 192-202. doi:10.4085/1062-6050-48.1.09

Needle, A. R., Kaminski, T. W., Baumeister, J., Higginson, J. S., Farquhar, W. B., & Swanik, C. B. (2017). The Relationship Between Joint Stiffness and Muscle Activity in Unstable Ankles and Copers. *J Sport Rehabil, 26*(1), 15-25. doi:10.1123/jsr.2015-0061

Needle, A. R., Lepley, A. S., & Grooms, D. R. (2017). Central Nervous System Adaptation After Ligamentous Injury: a Summary of Theories, Evidence, and Clinical Interpretation. *Sports Medicine, 47*(7), 1271-1288. doi:10.1007/s40279-016-0666-y

Needle, A. R., Palmer, J. A., Kesar, T. M., Binder-Macleod, S. A., & Swanik, C. B. (2013). Brain regulation of muscle tone in healthy and functionally unstable ankles. *J Sport Rehabil, 22*(3), 202-211.

Newham, D. J., Jones, D. A., Ghosh, G., & Aurora, P. (1988). Muscle Fatigue and Pain after Eccentric Contractions at Long and Short Length. *Clinical Science, 74*(5), 553-557. doi:DOI 10.1042/cs0740553

Newham, D. J., McPhail, G., Mills, K. R., & Edwards, R. H. (1983). Ultrastructural changes after concentric and eccentric contractions of human muscle. *Journal of the Neurological Sciences, 61*(1), 109-122.

Nilsson, J., Panizza, M., & Arieti, P. (1997). Computer-aided determination of the silent period. *Journal of Clinical Neurophysiology, 14*(2), 136-143.

Nosaka, K., & Clarkson, P. M. (1995). Muscle damage following repeated bouts of high force eccentric exercise. *Medicine and Science in Sports and Exercise, 27*(9), 1263-1269.

Palmieri-Smith, R. M., Hopkins, J. T., & Brown, T. N. (2009). Peroneal activation deficits in persons with functional ankle instability. *American Journal of Sports Medicine, 37*(5), 982-988. doi:10.1177/0363546508330147

Palmieri-Smith, R. M., Villwock, M., Downie, B., Hecht, G., & Zernicke, R. (2013). Pain and effusion and quadriceps activation and strength. *J Athl Train, 48*(2), 186-191. doi:10.4085/1062-6050-48.2.10

Rassier, D. E., MacIntosh, B. R., & Herzog, W. (1999). Length dependence of active force production in skeletal muscle. *J Appl Physiol (1985), 86*(5), 1445-1457. doi:10.1152/jappl.1999.86.5.1445

Rosager, S., Aagaard, P., Dyhre-Poulsen, P., Neergaard, K., Kjaer, M., & Magnusson, S. P. (2002). Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. *Scandinavian Journal of Medicine and Science in Sports, 12*(2), 90-98.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., Avanzini, G., Bestmann, S., . . . Grp, S. T. C. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology, 120*(12), 2008-2039. doi:10.1016/j.clinph.2009.08.016

Scholz, M. N., Bobbert, M. F., van Soest, A. J., Clark, J. R., & van Heerden, J. (2008). Running biomechanics: shorter heels, better economy. *Journal of Experimental Biology, 211*(Pt 20), 3266-3271. doi:10.1242/jeb.018812

Singer, J. D. G. A. B. (2009). Reflex and non-reflex elements of hypertonia in triceps surae muscles following acquired brain injury: implications for rehabilitation. *Disability and Rehabilitation, 23*(17), 749-757. doi:10.1080/09638280110060466

Stauber, W. T., Clarkson, P. M., Fritz, V. K., & Evans, W. J. (1990). Extracellular matrix disruption and pain after eccentric muscle action. *J Appl Physiol (1985), 69*(3), 868-874. doi:10.1152/jappl.1990.69.3.868

Thomas, N. M., Dewhurst, S., & Bampouras, T. M. (2015). Homogeneity of fascicle architecture following repeated contractions in the human gastrocnemius medialis. *Journal of Electromyography and Kinesiology, 25*(6), 870-875. doi:10.1016/j.jelekin.2015.08.007

Thomas, S., Reading, J., & Shephard, R. J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Canadian Journal of Sport Sciences, 17*(4), 338-345.

Trompetto, C., Avanzino, L., Marinelli, L., Mori, L., Pelosin, E., Roccatagliata, L., & Abbruzzese, G. (2012). Corticospinal excitability in patients with secondary dystonia due to focal lesions of the basal ganglia and thalamus. *Clinical Neurophysiology, 123*(4), 808-814. doi:10.1016/j.clinph.2011.06.033

Warren, G. L., Ingalls, C. P., Lowe, D. A., & Armstrong, R. (2002). What mechanisms contribute to the strength loss that occurs during and in the recovery from skeletal muscle injury? *Journal of Orthopaedic and Sports Physical Therapy, 32*(2), 58-64.

Warren, G. L., Lowe, D. A., Hayes, D. A., Karwoski, C. J., Prior, B. M., & Armstrong, R. B. (1993). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *Journal of Physiology, 468*, 487-499.

Werhahn, K. J., Kunesch, E., Noachtar, S., Benecke, R., & Classen, J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *Journal of Physiology, 517 (Pt 2)*(2), 591-597. doi:10.1111/j.1469-7793.1999.0591t.x

Whitehead, N. P., Weerakkody, N. S., Gregory, J. E., Morgan, D. L., & Proske, U. (2001). Changes in passive tension of muscle in humans and animals after eccentric exercise. *Journal of Physiology, 533*(Pt 2), 593-604. doi:10.1111/j.1469-7793.2001.0593a.x

Woittiez, R. D., Huijing, P. A., & Rozendal, R. H. (1983). Influence of muscle architecture on the length-force diagram of mammalian muscle. *Pflügers Archiv. European Journal of Physiology, 399*(4), 275-279. doi:10.1007/bf00652752

Yanagisawa, O., Sakuma, J., Kawakami, Y., Suzuki, K., & Fukubayashi, T. (2015). Effect of exercise-induced muscle damage on muscle hardness evaluated by ultrasound real-time tissue elastography. *Springerplus, 4*, 308. doi:10.1186/s40064-015-1094-4

Yu, J. G., Carlsson, L., & Thornell, L. E. (2004). Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochemistry and Cell Biology, 121*(3), 219-227. doi:10.1007/s00418-004-0625-9

Tables

Table 1

Descriptive Statistics of Pain Measures

Time	**Walk Pain Mean (± Std Dev)**	**Stand Pain Mean (± Std Dev)**	**Sit Pain Mean (± Std Dev)**
Pre	0.800 ± 0.646	0.854 ± 0.692	0.838 ± 0.666
Post	1.337 ± 0.867	1.329 ± 0.728	0.913 ± 0.604
24 Hours	2.208 ± 1.442*+	1.400 ± 0.973	1.183 ± 0.674
72 Hours	1.19583 ± 0.929	1.229 ± 0.972	1.075 ± 0.747

*Significantly different from baseline

+Significantly different from 72 hours

Table 2

Descriptive Statistics of PA and FL

	Pre	*Post*	*24 Hours*	*72 Hours*
Fascicle Length (± Std Dev) (cm)	9.117 ± 1.881	8.460 ± 2.498	8.436 ± 1.765	7.761 ± 1.708
Pennnation Angle (± Std Dev) (deg)	18.389 ± 3.153	19.137 ± 3.287	18.695 ± 2.724	20.083 ± 2.118

Table 3

Descriptive Statistics of Moment Arm

Technique	**Time**			
	Pre	*Post*	*24 Hours*	*72 Hours*
MA1 (± Std Dev) (cm)	2.603 ± 0.591	2.496 ± 0.724	2.502 ± 0.572	2.523 ± 0.635
MA1 Goni Corrected (± Std Dev) (cm)*	3.257 ± 0.860	2.858 ± 0.680	3.119 ± 1.005	3.271 ± 0.884
MA2 (± Std Dev) (cm)	2.668 ± 0.748	2.478 ± 0.639	2.499 ± 0.475	2.465 ± 0.593
MA2 Goni Corrected (± Std Dev) (cm)*	3.083 ± 0.811	3.047 ± 1.193	3.195 ± 0.758	3.387 ± 1.233
Manual (Pre-Only) (± Std Dev) (cm)†	5.266 ± 0.466	No difference between timpoints for any technique		
*Significantly different than MA1 and MA2, † Significantly different than MA1, MA2, MA1 Goni Corrected, MA2 Goni Corrected				

Table 4

Descriptive and ANOVA Statistics of Tendon Stiffness

Stiffness Range	**Moment Arm Technique**	**Time** (Mean ± Std Dev; N/cm)				*ANOVA Statistics*	
		Pre	*Post*	*24-hour*	*72-hour*	F-Value	P-Value
50% - 100% of MVIC Maximum	MA1 Raw	1826.41 ± 713.58	2117.04 ± 818.00	1915.66 ± 823.66	1944.62 ± 678.95	0.794	0.506
	MA1 Goni Corrected	1500.77 ± 677.66	1819.92 ± 706.5	1571.63 ± 671.22	1576.01 ± 704.07	1.032	0.391
	MA 2 Raw	1844.37 ± 960.12	1973.78 ± 631.35	1878.41 ±675.28	2058.32 ± 972.02	0.238	0.869
	MA2 Goni Corrected*	1588.37 ± 784.17	1779.40 ± 1026.82	1500.22 ± 654.81	1728.52 ± 1199.50	0.308	0.820
	Manual	1149.29 ± 300.58	1196.87 ± 339.43	1143.65 ± 331.58	1171.69 ± 224.31	0.251	0.860
Total MVIC	MA1 Raw	2123.98 ± 936.92	2516.94 ± 1017.64	2364.58 ± 1148.42	2258.74 ± 659.80	0.804	0.501
	MA1 Goni Corrected	2048.97 ± 1021.94	2388.91 ± 956.14	2096.29 ± 894.14	2101.63 ± 938.00	0.571	0.638
	MA 2 Raw	2132.19 ± 1277.90	2298.49 ± 707.47	2269.24 ± 849.31	2432.17 ± 1217.17	0.234	0.872
	MA2 Goni Corrected*	2505.14 ± 1565.34	2672.13 ± 1517.75	2500.70 ± 1510.08	2763.76 ± 2069.74	0.139	0.936
	Manual*	1354.14 ± 480.71	1468.27 ± 485.54	1415.53 ± 599.71	1389.74 ± 307.73	0.227	0.877

* Indicates Sphericity was violated. Greenhouse-Geiser correction applied for analysis

Table 5

Descriptive and ANOVA Statistics of Maximal Isometric Force

	Pre	Post	24 Hours	72 Hours	F-Value	P-Value	μ_p^2
Maximum Force (N) ± Std Dev	120.918 ± 44.656	120.323 ± 25.118	126.057 ± 39.997	133.761 ± 38.112	0.355	0.786	0.022

Figures

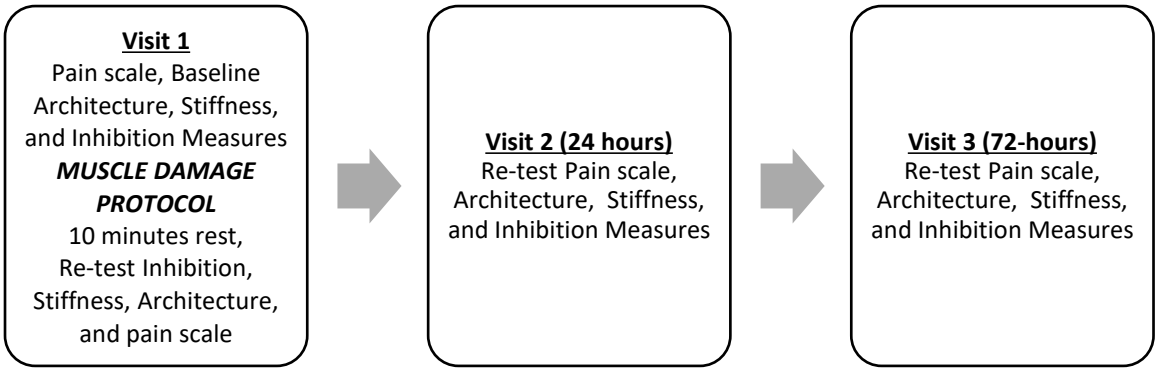


Figure 1: Timeline of Testing

Figure 2: Image of ultrasound placement
Superior view

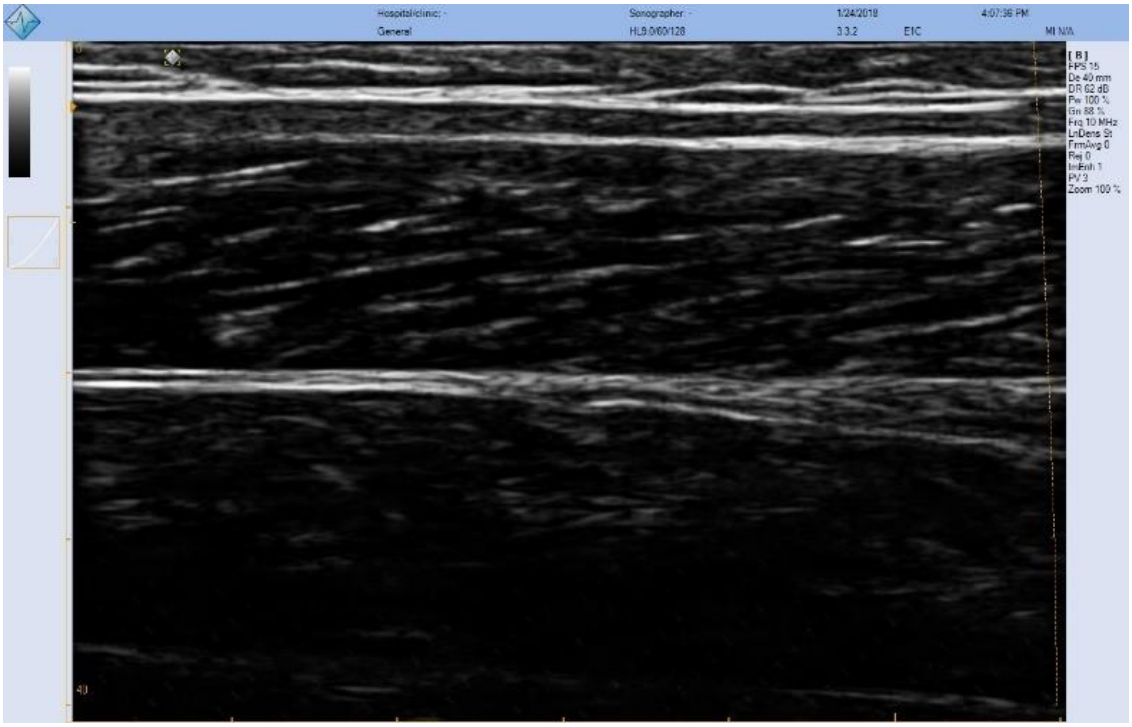


Figure 3: Ultrasound Image of MG

Figure 4: Foot images used to determine moment arm of ankle, Lateral View (top), Medial View (Bottom)

Figure 5: Image of tendon stiffness apparatus, Superior View (Top), Medial View (Bottom)

Figure 6: Placement of electro goniometer, Lateral View

Figure 7: Image of TMS apparatus

Figure 8: Muscle Damage Range of Motion: End Dorsiflexion(left), End Plantarflextion(Right), Medial View

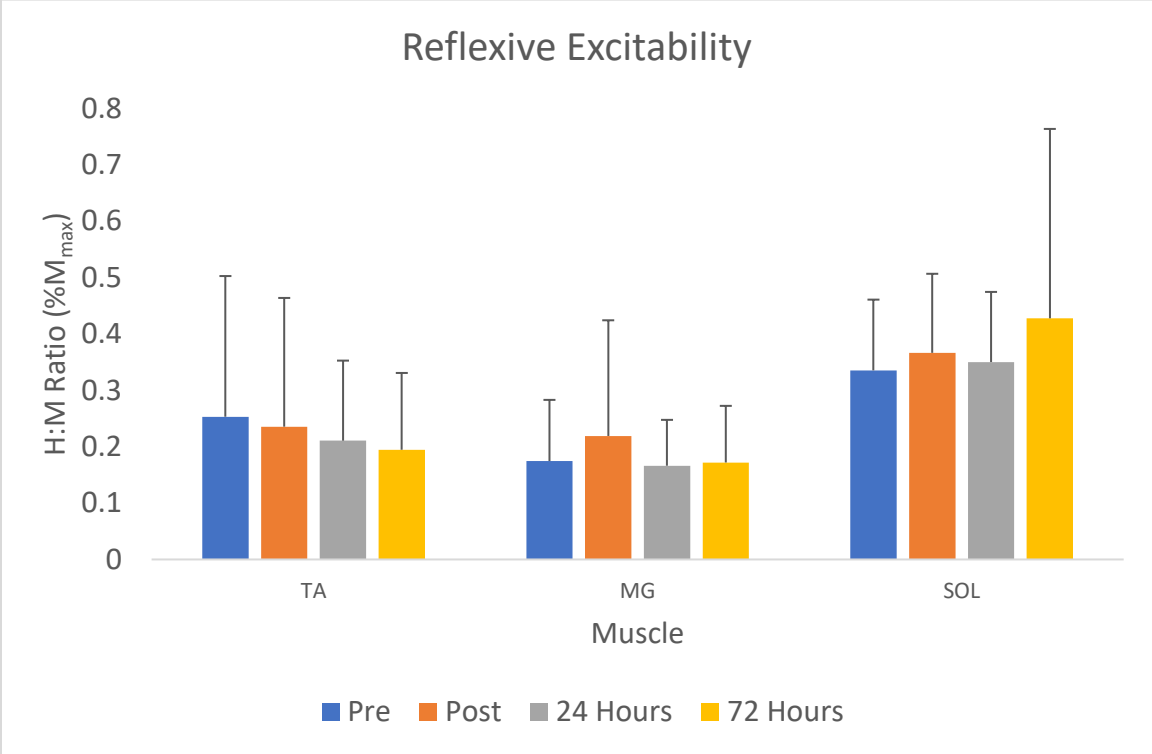


Figure 9: Descriptive statistics of H-Reflex

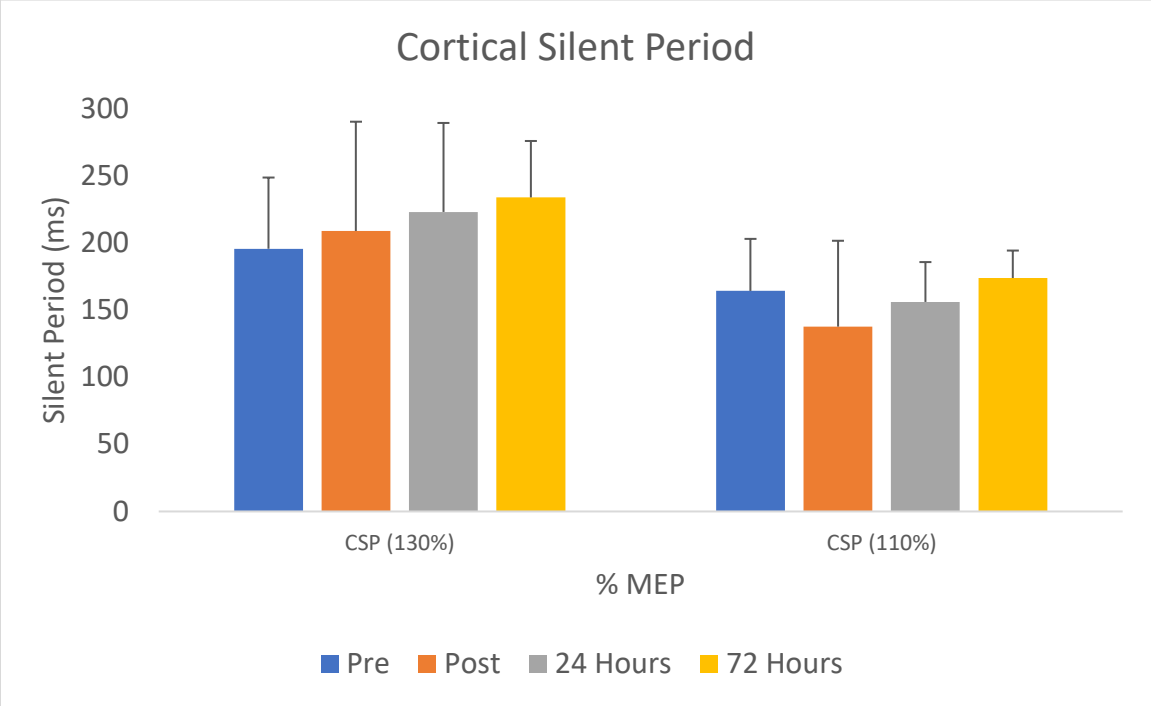


Figure 10: Descriptive Statistics of CSP

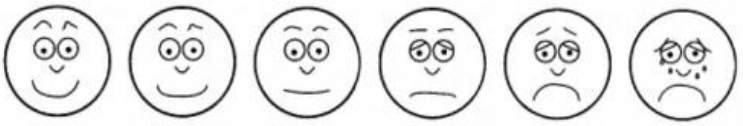
Appendix A

PAIN SCALES

Using a **vertical mark**, indicate your level of pain in your lower leg on the scales below:

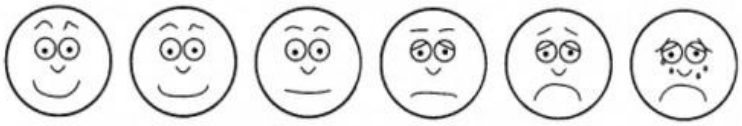
1) Please rate your current level of pain when **walking**

No pain at all — **Worst Pain Imaginable**



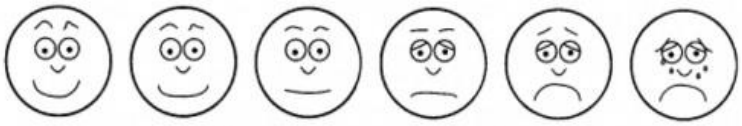
2) Please rate your current level of pain when **standing**

No pain at all — **Worst Pain Imaginable**



3) Please rate your current level of pain while **sitting**

No pain at all — **Worst Pain Imaginable**



Appendix B

Consent to Participate in Research
Information to Consider about this Research

Changes in Tendon Stiffness and Stiffness Regulation Following Exercise Induced Muscle Damage

Principal Investigator: John Mackall
Department: Health and Exercise science
Contact Information: mackalljw@appstate.edu
Faculty Advisor: Alan R. Needle, Ph.D.; needlear@appstate.edu

What is the purpose of this research?

Exercise-induced muscle damage (EIMD) describes the soreness that one experiences after performing weight training. When performing heavy lifting, the muscle experiences a small amount of damage and inflammation, which is a normal process allowing for processes that make the muscle and tendon stronger. However, the associated soreness is often a deterrent for many to continue exercise and resistance training.

In this study, we will look at how exercise-induced muscle damage affects how stiff your muscle becomes, as well as how your brain and nervous system change in response to the small amounts of inflammation. Understanding these factors will allow for us to better understand the effects of injury on the body, allowing for the development of better techniques to prevent and treat muscle damage and other types of injury.

Why am I being invited to take part in this research?

You are invited to participate because you are an able-bodied volunteer between the ages of 18-35 that has no current or recent injury in the lower extremity, have not participated in regimented training for the past three months, and have no current or past history of neurological disorder. If you volunteer to take part in this study, you will be one of about 15 people to do so.

Are there reasons I should not take part in this research?

You should not participate in this research if you have any current or past history of cardiac issues; seizure or epilepsy, or have an immediate relative with epilepsy; are hearing impaired or have ringing in your ears; have implanted medical devices including cochlear implants, metal in the brain or skull, an implanted neurostimulator, pacemaker, or a medication infusions device; are or may be pregnant; have a history of concussion within 6 months; experience recurrent bouts of fainting or syncope, or migraines; have a history of skull fracture or any skull abnormalities; or have a history of surgery to the brain or heart. The use of (or withdrawal from) several medications may also exclude you from participating in this study. The principal investigator will present you with a screening questionnaire and a list of medications that will determine your eligibility for this study. You will also not be allowed to participate in this study if you currently have a lower limb injury or are taking part in regimented physical activity.

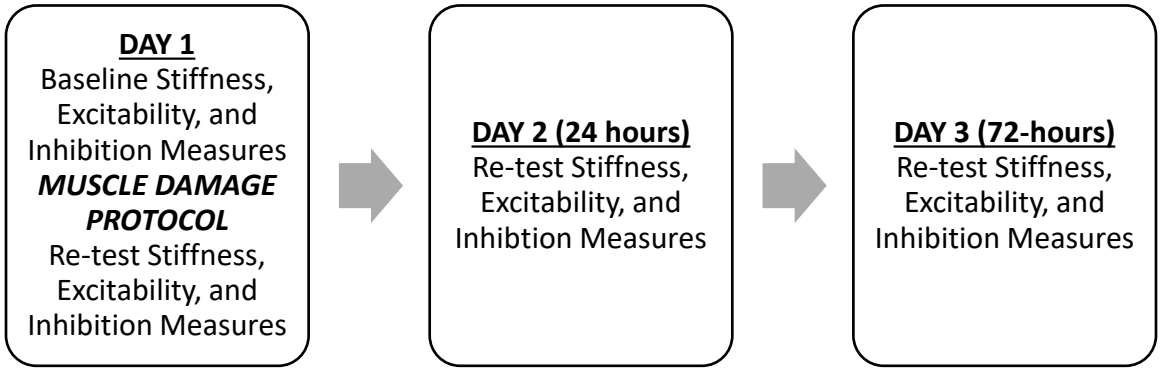
You should also not participate in this research if you have had an injury that prevented you from exercising within the last 3 months.

What will I be asked to do?

Complete participation in this study will entail a total of 3 sessions over the course of 72 hours. The first session will be approximately two hours comprised of baseline testing,

exercise protocol and post-tests. The second and third tests will occur 24 and 72 hours after the first session with a four-hour margin of error. Both session will be about 30 minutes and will be a retest of experimental outcomes.

Data Collection Timeline:



All testing will take place in the Appalachian State University Neuromuscular lab (Convocation Center Room 083). On the first day you will complete the physical activity readiness questionnaire (PAR-Q) and the transcranial magnetic stimulation screening questionnaire. We will then obtain baseline measurements of your tendon's stiffness and muscle architecture, spinal excitability, and cortical inhibition. These measures will take place in the same order and will be repeated immediately, 24-hours and 72-hours after completing a muscle damage protocol.

Tendon Stiffness and Muscle Architecture
Tendon stiffness and muscle architecture will be tested using ultrasound imaging with the sensor placed on your calf muscle. Ultrasound imaging is a safe technique with very few people commenting that it can produce a warming effect. Pictures will be taken of your bare foot and ankle. We will use these pictures to determine the moment arm of your foot, telling us about its ability to produce force. You will then lie on your front on an isokinetic dynamometer which is a padded table and on the end is a footplate run by an electric motor. You will then move into a full ankle stretch, followed by performing a heel raise against the sled as hard as you can as we look at your Achilles tendon and calf muscle using ultrasound.

Spinal Excitability
Electrical stimulation will be used to study the strength of the reflexes surrounding your ankle joint. A stimulating electrode will be placed behind your knee. Brief electrical pulses (less than half a second), will be applied while the muscle activity is recorded in your legs. The pulses will begin at a low intensity and will be increased in intensity as the muscle contraction in your leg is recorded. The intensity of the pulses will be increased until a maximal muscle contraction is observed. These pulses will produce a tingling sensation with a muscle contraction that will go away shortly after the stimulation.

Cortical Inhibition
Transcranial magnetic stimulation (TMS) will be used to administer magnetic pulses over your head. By measuring your muscles' responses to the pulses, we can determine the

strength of the connections between your brain and your ankle. You will be asked to wear a tight fitting cap so that measurements may be made on your head and will be provided earplugs to decrease the sound of the machine. You will then be familiarized with the magnetic stimulator which sends very short (less than half a second long) pulses through a large coil. The coil will touch the top of your head during the stimulation. Once familiarized, we will deliver one pulse every 5 seconds at different locations in approximately a 3-cm radius on your head. We will target the areas of your brain that control the muscles being measured. Up to 50 pulses may be delivered at varying intensities to determine your "motor threshold". Next you will receive 30 pulses at 110, 130 and 150% (10 at each intensity) of your motor threshold.

While TMS pulses are being delivered, you will be asked to remain seated with either your muscles relaxed or contracted slightly as if you stepping on a gas pedal. You will hear a click every time the TMS pulse is delivered. The TMS pulse will feel like a tap on your head and will cause twitching of your leg muscles. At higher intensities, the TMS pulse may cause your forehead or face to twitch.

EIMD Protocol
The protocol to induce muscle damage will be done by performing standing single-leg calf raises on the isokinetic dynamometer. Knees will be kept straight using knee braces. Your greatest force production value obtained from tendon stiffness will be used to determine the load for the EIMD protocol. Seventy-five percent of this value will be entered into the computer of the dynamomteter and you will then perform ten sets of ten repetitions of single-leg calf raise. Repetitions will only be eccentric (lowering contractions). A full range of motion will be done for each repetition and controlled for by the dynamometer. The range of motion will first be assessed prior to exercise with dynamometer. If you are unable to complete at least five full sets, we will discontinue your participation in this study.

What are possible harms or discomforts that I might experience during the research?

There will likely be muscle soreness that will occur after the exercise protocol. Soreness can begin as soon as immediately following exercise and can last as long as a week in some cases. Soreness should be limited to the calf muscles as this is the muscle being exercised. If the soreness persists for more than 10 days, you should follow-up with your physician or another qualified healthcare professional, as well as notifying the principal investigator.

There is a mild risk of skin irritation at the location where the muscle sensors are placed, but this will usually go away after the sensors are removed.

Rare cases of seizures during or immediately after TMS have been reported. While extremely rare utilizing the type of stimulation being used in this study, some cases have been reported in individuals without a previous history of seizure or neurological disease. This risk is potentially reduced by asking you some questions that might tell us about the risks of you experiencing seizure (*see TMS screening questionnaire)*. Individuals who have a history of seizures or have been diagnosed with epilepsy will be excluded from this study. Metal objects close to the coil may be affected by magnetic stimulation; we will therefore exclude individuals who have implants in their head. Some medications may contribute to an increased risk of seizure and will also result in exclusion from the study. You may feel twitches in the muscles of your arm, leg, or face during the magnetic

stimulation, but these twitches should not be painful. There is a possibility of headaches, scalp discomfort, or lightheadedness associated with TMS testing. If they occur, these effects are usually mild and short-lasting. In rare cases, fainting may occur.

While the use of TMS has the potential to help us better understand the neurological effects of exercise-induced muscle damage, you may opt out of this measure if you are uncomfortable with the risk level.

The investigator has discussed the risks associated with Transcranial Magnetic Stimulation (TMS) with me and, knowing these risks, I still wish to participate in this study.	Initial

During electrical stimulation, the pulses applied will cause a muscle twitch and tingling sensation shooting down the leg that may be uncomfortable; however, each pulse will last less than one second and every effort will be made to minimize the amount of pulses that must be applied. There may be some minor irritation of the skin around the site of the electrode following the experiment.

Are there any reasons you might remove me from the research?
There may be reasons we will need to remove you from the study, even if you want to stay in. If you experience an injury to either lower extremity between testing sessions, it will be at the discretion of the principal investigator whether to allow you to remain in the study. Failure to complete five sets of the EIMD Protocol will be grounds for termination. Additionally, if you experience any of the adverse reactions mentioned above, we will immediately terminate your participation in this study.

If you are pregnant you should not participate in this study. You should remove yourself from participation if you become pregnant, or suspect you are pregnant, at any point during the study. If you use pain or anti-inflammatory medications (e.g. Tylenol or NSAIDs) you will be removed from the study as these medications may modify your response to muscle damage. The ultrasound gel in rare occasions causes a topical rash and itching, if this occurs the testing will stop and you will be removed from the study.

What are possible benefits of this research?
There are no direct benefits to volunteers, and you are free to end your participation at any time. It is our hope that your participation in this project will improve our understanding of how muscle injury changes normal body function.

Will I be paid for taking part in the research?
There is no compensation for participating in the study.

What will it cost me to take part in this research?
You are responsible only for arranging transport to and from the laboratory for testing.

How will you keep my private information confidential?
Your identity will remain confidential and will not be revealed in any publications resulting from this work. All data will be stored on a secure long-term storage medium. The data will not have any identifiers linking information to you. The results of this study may be used for teaching, publications, or presentations at scientific meetings. If your individual results are discussed, your identity will be protected by using a study code rather than your name. Following completion of this project, the data will be destroyed or transferred to a long-

term storage medium for use during future research studies. Retained data will be stored on an encrypted secure server.

What if I get sick or hurt while participating in this research study?
In the rare event of an injury during testing, standard emergency procedures will be followed. There will be two investigators present at all testing sessions trained in emergency procedures and first aid. The testing facility is located within a few minutes of several agencies providing emergency treatment. If you need emergency care while you are at the research site, it will be provided to you. If you get hurt or sick when you are not at the research site, you should call your doctor or call 911 in an emergency. If your illness or injury could be related to the research, tell the doctors or emergency room staff about the research study, the name of the Principal Investigator, and provide a copy of this consent form if possible. Call the study's adviser, Alan R. Needle, Ph.D. (828-262-4039) as soon as you can. He needs to know that you are hurt or ill.

There are procedures in place to help attend to your injuries or provide care for you. Costs associated with this care will be billed in the ordinary manner, to you or your insurance company. However, insurance companies, Medicare, and Medicaid may not pay bills that are related to research costs. You should check with your insurance about this and talk to the Principal Investigator if you have concerns.

Whom can I contact if I have a question?
The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at 828-262-4039. If you have questions about your rights as someone taking part in research, contact the Appalachian Institutional Review Board Administrator at 828-262-2692 (days), through email at irb@appstate.edu or at Appalachian State University, Office of Research Protections, IRB Administrator, Boone, NC 28608.

Do I have to participate?
Your participation in this research is completely voluntary. If you choose not to volunteer, there is no penalty or consequence. If you decide to take part in the study you can still decide at any time that you no longer want to participate. You will not lose any benefits or rights you would normally have if you do not participate in the study.
.
I have decided I want to take part in this research. What should I do now?
If you have read this form, had the opportunity to ask questions about the research and received satisfactory answers, and want to participate, then sign the consent form and keep a copy for your records.

Participant's Name (PRINT) Signature
Date

Investigator's Name (PRINT) Signature
Date

Photography and Video Recording Authorization

With your permission, still pictures (photos) and/or video recordings taken during the study may be used in research presentations of the research findings. Please indicate whether or not you agree to having photos or videos used in research presentations by reviewing the authorization below and signing if you agree.

Authorization

I hereby release, discharge and agree to save harmless Appalachian State University, its successors, assigns, officers, employees or agents, any person(s) or corporation(s) for whom it might be acting, and any firm publishing and/or distributing any photograph or video footage produced as part of this research, in whole or in part, as a finished product, from and against any liability as a result of any distortion, blurring, alteration, visual or auditory illusion, or use in composite form, either intentionally or otherwise, that may occur or be produced in the recording, processing, reproduction, publication or distribution of any photograph, videotape, or interview, even should the same subject me to ridicule, scandal, reproach, scorn or indignity. I hereby agree that the photographs and video footage may be used under the conditions stated herein without blurring my identifying characteristics.

Participant's Name (PRINT)	Signature	Date

Appendix C

Data Collection Sheet

NAME:_____ DATE:_____

HEIGHT:_____in. WEIGHT:_____lbs. AGE:_____

PHYSICIANS NAME:_____ PHONE:_____

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

	Questions	Yes	No
1	Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?		
2	Do you feel pain in your chest when you perform physical activity?		
3	In the past month, have you had chest pain when you were not performing any physical activity?		
4	Do you lose your balance because of dizziness or do you ever lose consciousness?		
5	Do you have a bone or joint problem that could be made worse by a change in your physical activity?		
6	Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?		
7	Do you know of any other reason why you should not engage in physical activity?		

If you have answered "Yes" to one or more of the above questions, consult your physician before engaging in physical activity. Tell your physician which questions you answered "Yes" to. After a medical evaluation, seek advice from your physician on what type of activity is suitable for your current condition.

Appendix D

Appalachian State University
Transcranial Magnetic Stimulation Screening Questionnaire

We need to ask you about these things to ensure you are safe. These help us identify risk factors may be associated with seizure during TMS procedures.

1.	Do you have **epilepsy** or have you ever had a convulsion or seizure?	□ Yes	□ No
2.	Do you have any *immediate* family members with a history of epilepsy?	□ Yes	□ No
3.	Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s)?	□ Yes	□ No
4.	Have you ever had head trauma that was diagnosed as a concussion or was associated with loss of consciousness? If yes, how long ago was your most recent concussion?	□ Yes	□ No
5.	Do you have any hearing problems or ringing in your ears?	□ Yes	□ No
6.	Do you have cochlear implants?	□ Yes	□ No
7.	Are you pregnant or is there a chance you might be?	□ Yes	□ No
8.	Do you have metal in the brain, skull, or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, specify the type of metal.	□ Yes	□ No
9.	Do you have an implanted neurostimulator (e.g. DBS, epidural/subdural, VNS)?	□ Yes	□ No
10.	Do you have a cardiac pacemaker or intracardiac lines?	□ Yes	□ No
11.	Do you have a medication infusion device?	□ Yes	□ No
12.	Do you frequently suffer from migraine headaches?	□ Yes	□ No
13.	Do you have a history of skull fracture or any present skull abnormalities?	□ Yes	□ No
14.	Have you ever had surgery to the brain or heart?	□ Yes	□ No
15.	Are you taking any medications?	□ Yes	□ No
	If so, do they match any of the medications listed on the opposite side of this page?	□ Yes	□ No
16.	Did you ever undergo TMS in the past?	□ Yes	□ No
	If so, were there any problems?	□ Yes	□ No
17.	Did you ever undergo MRI in the past?	□ Yes	□ No
	If so, were there any problems?	□ Yes	□ No

List of Potentially Hazardous Drugs for TMS
CLASS A

Medications

Amitriptyline	**Chlorpromazine**	**Clozapine**	**Doxepine**
Foscarnet	**Ganciclovir**	**Imipramine**	**Ketamine**
Maprotiline	**Nortriptyline**	**Ritonavir**	**Theophylline**

Recreational Drugs

Alcohol	**Amphetamines (i.e. methamphetamine)**	**Cocaine**	**MDMA** *(ecstasy)*
Phenycyclidine *(PCP, angel dust)*	**Gamma-Hydroxybutyrate (GHB)**	**Lysergic acid diethylamide (LSD)**	

CLASS B

Ampicillin *(Ominpen, Polycillin, Principen)*	**Anticholinergics** *(i.e. Atrovent, Albuterol, Combivent, DuoNeb)*	**Antihistamines** *(i.e. Allegra, Claritin, Benadryl)*	**Aripiprazole** *(Abilify)*
BCNU *(Carmustine)*	**Bupropion** *(Wellbutrin, Aplenzin)*	**Cephalosporins** *(Cephalosproium)*	**Chloroquine** *(Aralen)*
Chlorambucil *(Leukeran)*	**Ciproflaxacin**	**Citalopram** *(Celexa, Cipramil)*	**Cyclosporin** *(USAN, BAN)*
Cytosine arabinoside *(Cytarbine)*	**Duloxetine** *(Cymbalta, Yentreve)*	**Fluoxetine** *(Prozac)*	**Fluphenazine** *(Prolixin)*
Fluvoxamine *(Luvox)*	**Haloperidol** *(Haldol)*	**Imipenem** *(Primaxin)*	**Isoniazid** *(Laniazid, Nydrazid)*
Levofloxacin *(Levaquin)*	**Lithium** *(Lithoboid, Eskalith)*	**Mefloquine** *(Lariam)*	**Methotrexate** *(Trexall, Rhumatrex)*
Metronidazole *(Flagyl)*	**Mianserin** *(Bolvidon, Norval, Tolvon)*	**Mirtazapine** *(Remeron, Avanza, Zispin, Reflex)*	**Paroxetine** *(Aropax, Paxil)*
Olanzapine *(Zyprexa, Zydis, Relprevv)*	**Penicillin**	**Pimozide** *(Orap)*	**Quetiapine** *(Seroquel)*
Reboxetine *(Edronax, Vestra)*	**Risperidone** *(Risperdal)*	**Ritalin, Ephedrine, or other Sympathomimetics**	**Sertraline** *(Zoloft)*
Venlafaxine *(Effexor)*	**Vincristine** *(Oncovin)*	**Ziprasidone** *(Geodon)*	

Additionally, you should *not* participate in this study if you are undergoing symptoms of **withdrawal** from *alcohol, barbiturates, benzodiazepines, meprobamate, or chloral hydrate.*

If you are on any other medications (other than listed above) that you are concerned may increase the risk for adverse events related to Transcranial Magnetic Stimulation, please let the principle

For Investigator Use Only:
If subject answered yes for any question, explain below:

_____

_____

investigator know. Additionally, the principal investigator can provide you with a list of medications, their uses, and trade names if you are unclear of anything on the above list.

For questionnaire administrators:

- Any "Yes" responses to questions must be followed-up with the primary investigator of the current study.
- For question #1, 2, 5, 6, 7, 8, 9, 10, 11, 13, 14, or 15 – any answer of "Yes" indicates immediate exclusion from the study.
- For question #3 & #12 – the primary investigator will follow-up and determine if these represent isolated incidents or are frequent occurrences. If frequent (occurring more than 4 times per year), the subject will be excluded; however, if rare, subjects will be informed of the risks of the study and given the option to participate.
- For question #4 – the primary investigator will follow-up and determine if the subject has been symptom free from their concussion for at least 6 months, and did not suffer any symptoms of post-concussion related syndrome. If these criteria are not met, the subject will be excluded.
- For questions #16 and 17 – the primary investigator will follow-up and determine the complication of previous testing and determine if that risk is still applicable to the current protocol (i.e. claustrophobia in an MRI machine will not lead to study exclusion). If the complication is still a possible risk, the subject will be excluded.

Vita

John William Mackall was born in Silver Spring, Maryland, to John Ford and Rhonda Michelle Mackall. In 2014, he graduated from Salisbury University in Maryland with a Bachelor of Science in Exercise Science and a minor in Business Administration. He began his graduate education at Appalachian State University in the fall of 2015. While at Appalachian State University, he earned degrees in Engineering Physics and Exercise Science.

Mr. Mackall is a member of Sigma Pi Sigma and Institute for Electrical and Electronics Engineers. He resides in Boone, NC.